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RBP927 PROTEIN:

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    |  10     |  20     |  30     |  40
MHKEEHEVAV LGAPPSTILP RSTVINIHSE TSVPDHVVWS

    |  50     |  60     |  70     |  80
LFNTLFLNWC CLGFIAFAYS VKSRDRKMVG DVTGAQAYAS

    | 110     | 120     | 130     | 140   145
TAKCLNIWAL ILGILMTIGF ILSLVFGSVT VYHIMLQIIQ EKRGY
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β-gal-RBP927 HYBRID PROTEIN IN CLONE #11:

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    |  10     |  20     |  30      37
MTMITPSAQL TLTKGNKSWS STAVAAALEL VDPPGCR....--RBP927
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(57) Abstract

The present invention is directed to pharmaceutical compositions and methods for the treatment of viral infections. Interferon-inducible 1-8 polypeptides are employed to inhibit viral structural gene expression and hence viral replication. Also provided are methods for assessing the efficacy of interferon therapy in patients.

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INHIBITION OF RETROVIRAL EXPRESSION BY INTERFERON-INDUCED CELLULAR GENES AND PROTEINS

Background of the Invention

This invention relates generally to compositions and uses of interferon-induced 1-8 gene family genes, and polypeptides encoded by those genes in the treatment of retroviral diseases. The polypeptides may be used to inhibit expression of retroviral proteins in infected cells or assayed to assess the efficacy of interferon therapy of retroviral disease. The genes may be incorporated into expression vectors or introduced into host genomes by shuttle vectors to increase cellular production of interferon-induced 1-8 gene family proteins or polypeptides.

Retroviral infections (i.e., infections by viruses which use reverse transcription to replicate) cause significant morbidity and mortality in humans and other animal species. Human T-Cell Lymphotropic Virus (HTLV-I) is a retrovirus associated with leukemia in humans (Poiesz et al., Proc. Natl. Acad. Sci. U.S.A., 77:7415 (1980)) and HTLV-II is associated with hairy cell leukemia in humans (Kalyanaraman et al., Science 218:571 (1982). Simian T Lymphotropic Virus-I (STLV-I) is associated with leukemia in numerous species of Asian and African primates (Miyoshi, et al. Gann 73:848 (1982) and Homma et al., Science 225:716 (1982)). Bovine leukemia virus (BLV) is associated with persistent lymphocytosis and eventually leukemia in cattle, (Ferrer et al., Cancer Res. 34:893 (1974)), visna virus is associated with a chronic degenerative disease in sheep (Haase, Nature 322:130-136 (1986), and feline immunodeficiency virus (FIV) causes a wide variety of symptoms in cats. Most notably, the human immunodeficiency virus, HIV-1, the causative agent of the

acquired immune deficiency syndrome (AIDS), and HIV-2, are retroviruses.

Following infection of a cell, retroviral RNA serves as a template for viral reverse transcriptase to produce DNA that encodes viral proteins. The DNA is transcribed by host mechanisms to mRNA. Viral proteins are then synthesized by normal host cell mechanisms. The expression of many retroviral proteins is regulated by the binding of virally encoded proteins to specific viral mRNA sequences.

The synthesis of many retroviral proteins in infected host cells as well as viral replication is dependent upon a small nuclear regulatory protein encoded by a gene termed rev (formerly referred to as art/trs). See, e.g., Sodroski et al., Nature 321:412-417 (1986), Feinberg et al., Cell 46:807-817 (1986), and Terwilliger et al., J. Virol. 62:655-658 (1988). Rev is a small protein of about 116 amino acids, and is produced by six doubly or triply spliced messenger RNAs (mRNAs). Sadie et al., Proc. Natl. Acad. Sci. USA 85:9224-9228 (1988), Felber et al., J. Virol. 64:3734-3741, (1990) and Schwartz et al., J. Virol. 64:2519-2529 (1990). Rev is primarily accumulated in the nucleoli of the infected cell, Cullen et al., J. Virol. 62:2498-2501 (1988) and Felber et al., Proc. Natl. Acad. Sci. USA 86:1495-1499 (1989), and apparently regulates the export of viral structural mRNAs from the nucleus to the cytoplasm. Felber et al., Proc. Natl. Acad. Sci. USA 86:1495-1499 (1989); Hadzopoulou-Cladaras et al., J. Virol. 63:1265-1274 (1989); Pavlakis et al., in Human Retroviruses, Ed. J. Groopman et al., Alan R. Liss, Inc. NY, 1990, pp 141-152; and Malim et al., Nature 338:254-257 (1989).

Rev function is mediated through a unique cis-acting element of approximately 200 nucleotides, known as the Rev-responsive element, or RRE, found in the env region of HIV-1. A direct interaction between Rev and RRE has been demonstrated by in vitro binding experiments. Zapp et al., Nature 342:714-716 (1989); Daly et al., Nature 342:816-819 (1989); and Cochrane et al., Proc. Natl. Acad. Sci. USA 87:1198-1202

(1990). Binding of Rev to retroviral mRNA may permit the mRNA to be transported out of the nucleus into the cytoplasm in unspliced form. Alternatively, Rev may bind to RRE and promote the dissociation of components of the splicing machinery and pre-mRNA. (For a review, see, Rosen and Pavlakis, AIDS 4:499-509 (1990)). Other related viruses, such as HIV-2 and SIV, contain RRE elements and express functional Rev proteins. The analogous RRE elements of other lentiviruses, such as the Rex-responsive element (RXRE) of HTLV-I, HTLV-II and BLV, appear to act in a manner similar to that of HIV-1.

In addition to viral factors, cellular activators and repressors are important for viral expression. The average time interval between acquisition of HIV infection and onset of AIDS is approximately 8 to 11 years (Lifson et al., J. Infect. Dis. 158:1360 (1988)), suggesting that HIV replication is at least partially restricted by host factors. Indeed, the transcriptionally active population of HIV-infected cells is approximately 100- to 1000-fold less than the total number of infected leukocytes. Harper et al., Proc. Natl. Acad. Sci. USA 83:772 (1986). The mechanisms responsible for this potent restriction in virus replication are poorly understood, but have been postulated to involve factors induced in infected cells by exposure to cytokines such as interleukine-1 (IL-1), IL-2, tumor necrosis factor-α, GM-CSF, and interferon.

In humans, variable levels of interferon production have been reported in HIV-infected patients. Interferon has been found in the sera of patients with late stage disease (Preble et al., Ann. N. Y. Acad. Sci. 437:65 (1985)), and has been proposed to be an index of poor prognosis. Vadhan et al., Cancer Res. 46:417 (1986). Murray et al., N. Engl. J. Med. 313:1504 (1985). Anderson et al., Int. Conf. AIDS (1989). Preble et al., AIDS and Infections in Homosexual Men, 2d ed., P.Ma and D. Armstrong eds. Butterworths, Boston, MA (1989). Interestingly, recombinant interferon-α treatment of Kaposi's sarcoma and AIDS reduces levels of p24 gag antigen in

serum and the frequency of virus isolation from blood leukocytes. De Wit et al., Lancet 2:1214 (1988) and Lane et al., Lancet 2:1218 (1988). Moreover, the antiviral effects of interferon against HIV-1 infection, particularly in reducing HIV replication in interferon-treated human target cells in vitro, are well established. See, e.g., Francis et al., AIDS Res. Hum. Retrov., 8:199-207 (1992); Friedman, J. Exp. Pathol., 5:49-51 (1990); Ho et al., Lancet, 1:602-604 (1985); Kornbluth et al., Clin. Immunol., Immunopathol., 54:200-19 (1990); Meltzer et al., Annu. Rev. Immunol., 8:169-94 (1990); and Poli et al., Science, 244:575-7 (1989). HIV-1 infection of primary cells and cell lines in vitro does not, however, lead to interferon induction. Dubreuil et al., Virology, 179:388-94 (1990), and Gendelman et al., J. Exp. Med., 172:1433-42 (1990). Peripheral blood mononuclear cells from infected individuals have a diminished capacity to produce interferon in response to viruses that typically induce interferon production (Lopez et al., J. Infect. Dis. 148:962-966, (1983) and Voth et al., J. Immunol. 144:970-975, (1990)). Hence, one hypothesis has been that HIV may thwart any natural host interferon protection by suppressing interferon production.

Even though interferon is being used in clinical trials to treat AIDS patients, based on the inhibitory effects of interferon in vitro, very little is known about the mechanism(s) responsible for these inhibitory effects. In general, at least some of the many actions exerted by the interferons (α, β and γ), including regulating cell growth and immune responses, are effected by gene activation. The interferons have been reported to induce upwards of 20 genes. Kerr and Stark, FEBS Lett. 285:194-8 (1991). Host cell synthetases and kinases are among the proteins induced by interferon.

Interferons also induce the expression of a group of genes and pseudogenes known as the "interferon-induced 1-8 gene family." See, e.g., Friedman et al., Cell 38:745-755 (1984), and Kelly et al., Eur. J. Biochem. 153:367-371 (1985).

This gene family consists of at least three functional genes and one or more pseudogenes. Lewin et al., Eur. J. Biochem., 199:417-23 (1991). Three members of the family (1-8U, 1-8D and 9-27) are linked and have been isolated on a single fragment of human genomic DNA. The 1-8U and 9-27 genes are highly inducible with interferon, whereas the 1-8D gene reportedly shows a reduced response to interferon. Although this gene family has been known for some time, the function of the proteins encoded thereby has previously not been ascertained.

Despite intensive research efforts, AIDS and other retroviral infections have proven to be relatively resistant to treatment. Presently approved methods of HIV treatment employ synthetic pharmaceuticals, such as 3'-azido-2',3'-dideoxythymidine (AZT) and 2',3'-dideoxyinosine (ddI), to block reverse transcriptase. While these medications have been shown to decrease morbidity and increase life expectancy of HIV infected individuals, neither treatment is curative. Also, these drugs have serious side-effects that are dose limiting and preclude treatment of many patients.

Treatment protocols exploiting host natural defense mechanisms may become preferable or complementary to the presently available regimens for treatment of retroviral infections. Although interferon therapy is under study, it has many shortcomings, including side effects, and may be of limited efficacy in AIDS patients. What is needed in the art is an effective means to inhibit retroviral replication in an infected host with minimal potential for causing significant toxicity to host cells. Quite surprisingly, the present invention fulfills this and other related needs.

Summary of the Invention

The present invention provides pharmaceutical compositions for the inhibition of a retroviral infection, such as HIV-1, HIV-2, HTLV-I, or BLV, for example, in cells of a human or animal host. The compositions comprise a polypeptide encoded by an interferon-inducible 1-8 gene family member and which is capable of binding to a retroviral Rev-responsive element, and a pharmaceutically acceptable carrier. Preferred polypeptides for use in the pharmaceutical compositions are encoded by genes 9-27 or 1-8U of the 1-8 gene family or biologically active fragments or mutants thereof.

Accordingly, the invention also provides methods of inhibiting a retroviral infection in an infected host. A polypeptide encoded by an interferon-inducible 1-8 gene family member or mutant thereof, such as RBP927 or 1-8U, is administered to the host in an amount effective to inhibit the infection. The infection may be caused by an HIV virus, such as HIV-1 or -2, or the polypeptides may be administered prophylactically to inhibit an infection from becoming established. In cases of well established infection the polypeptide may be administered to the host in multiple doses over a prolonged period of time. The polypeptides may also be used in conjunction with other retroviral treatments to inhibit viral replication. Typically the method of administration to the host will be parenteral, e.g., intravenous or intramuscular. The polypeptides and pharmaceutical compositions thereof can also be used in extracorporeal treatment of a host's cells, and in methods of inhibiting retrovirus replication in cell cultures. Furthermore, the invention also provides means to screen for additional compounds or mutants thereof which potentiate the retroviral-inhibiting effect of a subject polypeptide of the interferon-inducible 1-8 family, or which compete with the polypeptide to inhibit the retroviral Rev protein from binding to the RRE.

In additional embodiments eukaryotic expression vectors are provided which comprise a member of the interferon-induced 1-8 gene family, or a mutant thereof, operably linked to a promoter. These expression vectors are useful in cells, such as those of an infected host, to provide for or to augment a host's ability to express retroviral inhibitory polypeptides of the invention. The cells may be cotransfected with a vector encoding a different antiviral polypeptide, such as RevBL a transdominant Rev protein mutant.

In yet other embodiments the invention provides methods of assessing the efficacy of interferon therapy in a retroviral infected patient. The level of a expression product of an interferon-induced 1-8 gene family member, such as mRNA or protein of, e.g., RBP927 or 18U, is quantitated in cells or samples thereof from the patient exposed to interferon and compared to the level of the expression product in cells not exposed to interferon therapy. Interferon therapy can be predicted as at least partially efficacious when the levels of the interferon-inducible 1-8 gene family member expression product are enhanced as a result of exposure to the interferon.

Brief Description of the Figures

Fig. 1A illustrates the selective binding of RRE probes and a human β-globin probe to plaques of 12 recombinant phage clones, where clone No. 11 binds to intact RRE (RRE_{330}) but not to probe RREΔ345 which lacks 82 nucleotides of RRE comprising hairpin loops 3, 4 and 5, nor to the globin RNA probe.

Fig. 1B is the amino acid sequence of RBP927 and of the β-gal-RBP927 hybrid protein produced by clone no. 11, where the first 27 amino acids of RBP927 (underlined) were replaced by the 37 amino-terminal amino acids of the lacZ gene. The sequence of the insert begins with Ser_{28} and

consists of the 118 carboxy-terminal amino acids of the 927 protein.

Fig. 1C shows the nucleotide and amino acid sequences of the thrombin cleavage site and six additional amino-terminal amino acids of the GST-927 fusion protein.

Fig. 1D depicts the eukaryotic expression plasmids for RBP927, 18-U and 18-D, where LTR is the HIV-1 LTR promoter and shaded boxes represent the coding regions.

Fig. 2A illustrates the results of competition binding experiments of RBP927 to labeled RRE330 RNA or RREΔ345 RNA in the presence of increasing amounts of unlabeled competing E. coli tRNA.

Fig. 2B illustrates the results of competition binding experiments of RBP927 binding to labeled RRE330 transcripts in the presence of increasing amounts of unlabeled competitor nucleic acids: RRE330 RNA, or RREΔ345 RNA, globin RNA, E. coli tRNA, single-stranded DNA, or double-stranded DNA.

Fig. 3 shows the identification of RBP927 in human cells by immunoprecipitation using rabbit antisera (Fig. 3A), or by immunofluorescence (Fig. 3B).

Fig. 4A is a Western blot analysis of the inhibition of HIV-1 protein expression by RBP927, where filters were probed with HIV-1 patient serum, and the lanes are as follows: lane M, H9/HXB2 lysate; lane 1, pHXB2 alone; lane 3, pHXB2 and 1 μg of pL927; lane 3, pHXB2 and 5 μg of pL927; and lane 4, pHXB2 and 10 μg of pL927.

Fig. 4B is a quantitation of p24gag protein of the samples shown in Fig. 4A, with the means and standard deviation of four independent experiments shown.

Fig. 4C is a Northern blot analysis of RNA from transfected cells using a ^{32}P-labeled DNA probe from the HIV-LTR region, where U is unspliced, I is intermediate spliced, and S is small multiple spliced, viral mRNA species.

Fig. 5 illustrates the inhibition of HIV-1 gag protein expression by RBP927, where Fig. 5A is the Western blot analysis of proteins extracted from cells transfected

with the gag expressing plasmid pCgagA2 and the Rev expressing plasmid pL3crev, and increasing amounts of pL927, where lane M is H9/HXB2 lysate, lane 1 is pCgagA2 alone, lane 2 is pCgagA2 and pL3rev, and lanes 3-6 are pCgagA2 and pL3rev with increasing amounts of RBP927 (0.5, 2.5, 5.0 and 10 μg, respectively). Fig. 5B is the quantitation of gag production using p24 gag antigen capture assay, (and Luciferase activity) where the means and standard deviation of five independent experiments are shown and gag production in the presence of Rev was taken as 100%.

Fig. 6A illustrates that RBP927 inhibits only Rev-dependent protein expression, as shown for HLtat cells transfected with different gag expressing plasmids (pCgagA2 requires Rev for gag expression and p37M1234 has a gag coding region with mutations which allow high levels of Rev-independent gag expression) in the presence of increasing amounts of pL927.

Fig. 6B is a Western blot analysis of p19gag expression in HLtat cells transfected with pNLp19 and increasing amounts of pL927. Expression of p19gag was not significantly affected by increasing amounts of pL927.

Figs. 6C and 6D are Northern and Western blot analysis, respectively, of HLtat cells transfected with 5 μg pCMVp19R and increasing amounts of pL927, as indicated, where total RNA was isolated and probed with an RSV probe in the Northern analysis, and anti-p19 gag antisera was used for the Western blot.

Figs. 7A and 7B show the inhibition of HIV-1 p24 gag expression by RBP927 and the effects of related interferon-inducible proteins 18D and 18U, where HLtat cells were transfected with 2 μg of the HIV-1 proviral clone pHXB2 (Fig. 7A) and 1 μg of the gag expression plasmid pCgagA2 (Fig. 7B), in the presence of increasing amounts of pL927, pL18U, pL18D together with a control luciferase encoding plasmid. The mean and standard deviation shown from three independent experiments are shown.

Fig. 8 illustrates inhibition of HTLV-1 proteins by RBP927. HLtat cells were transfected with CS-HTLV-I and increasing amounts of pL927. Expression of HTLV-1 proteins was inhibited by the presence of RBP927.

Fig. 9 illustrates inhibition of $p24^{gag}$ expression in HIV-1 expressing cells that have been co-transfected by pL927, pLRevBL, or both.

Fig. 10 illustrates the effect of low level administration of pL927 to HIV-1 expressing cells co-transfected with pLRevBL.

Fig. 11 illustrates the effect of low level administration of pLRevBL to HIV-1 expressing cells cotransfected with Pl927.

Figs. 12A-B illustrate the effect on HIV-1 protein expression by cotransfection of HIV-1 expressing cells by pL927 and either pL18U or pL18D.

Fig. 13 illustrates RBP927 frameshift and deletion mutants and their associated inhibition of HIV-1 protein expression.

Fig. 14 illustrates point mutations of RBP927 and associated ability to inhibit expression of HIV-1 proteins.

Fig. 15 illustrates activation of RBP927 mRNA expression in peripheral blood mononuclear cells infected with HIV-1.

Description of the Specific Embodiments

The present invention provides polypeptides useful in pharmaceutical compositions and methods for inhibiting retroviral infection of host cells. The polypeptides useful herein are encoded by an interferon-induced 1-8 gene family member or mutant thereof. As used herein, the term "interferon-inducible 1-8 gene family member or mutant thereof" is meant to indicate a nucleic acid sequence of the interferon-inducible 1-8 gene family as described below or a

mutant of any of those genes. The mutation will often be site-directed. Often, the mutation will result in a substitution of a gly-ala dipeptide for a dipeptide in RBP927 protein (SEQ ID NO:2). The mutations will generally be made in the region of amino acids 35-42 of SEQ ID NO:2. Alternatively, site-directed mutations may be made in other interferon-inducible 1-8 gene family members, such as 1-8U. Deletion, insertion and frame shift mutations are also contemplated. Such mutations may be made by methods well known to those of skill in the art. These methods are described in, e.g., Sambrook et al, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, 1989, incorporated herein by reference. Unless otherwise indicated, it will be understood that the terms "interferon-inducible 1-8 gene", "1-8 gene", or the like will encompass wild-type and mutant interferon-inducible 1-8 gene family genes. Likewise, "interferon-inducible 1-8 polypeptide (or protein)", "1-8 polypeptide", or the like will be understood to include proteins and polypeptides encoded by wild-type and mutant 1-8 genes or fragments thereof.

One aspect of the invention is based upon the surprising discovery that polypeptides encoded by genes of the interferon-induced 1-8 gene family bind to the RRE region of retroviral mRNA, and inhibit retroviral protein synthesis and/or assembly of retroviral structural proteins. Because synthesis of viral proteins is inhibited, viral replication is also inhibited. As further described herein, the interferon-induced polypeptides may block or otherwise interfere with the binding of the Rev protein to the RRE. Accordingly, the up-regulation of the retroviral mRNA expression typically associated with Rev is inhibited.

The interferon-induced 1-8 gene family has been previously described. See generally, Friedman et al., Cell 38:745-755 (1984), Kelly et al., Eur. J. Biochem. 153:367-371 (1985), Reid et al., Proc. Natl. Acad. Sci. USA 86:840-844 (1989); Porter et al., EMBO J. 7:85-92 (1988); and Lewin et al., Eur. J. Biochem., 199:417-23 (1991), each of which is

incorporated herein by reference. The interferon-induced 1-8 gene family refers to a group of genes which is inducible by both α- and β-interferons. At least three functional genes, designated 1-8U, 1-8D and 9-27, and one or more pseudogenes have been identified as members of the 1-8 gene family. Three members of the family (1-8U, 1-8D and 9-27) are linked and have been isolated on a single fragment of human genomic DNA. The 1-8U and 9-27 genes are highly inducible with interferon, whereas 1-8D shows a reduced response to interferon. The function of this group of proteins has previously not been ascertained.

Members of the interferon-induced 1-8 gene family are characterized by an identical 13bp ISRE (Interferon-stimulable response element) in their promoter region that responds to both alpha and gamma interferons. All three members of the family share a region of 45 amino acids (50 to 94 for RBP927) that is completely identical and also share approximately 75% overall homology. They also contain a potential "leucine zipper" motif close to the carboxy terminus and three potential phosphorylation sites. Lewin et al., Eur. J. Biochem. 199:417-423 (1991). There is a well described family of interferon inducible genes in the mouse (termed Mx family) members of which have been shown to exhibit antiviral activities (Chang et al., Arch. Virol. 117:1-15 (1991). Also in many other mouse chromosomes (1, 2, 3, 12, 16 and 17) other interferon stimulated genes have been identified some which established and some with unknown functions. Choubey et al. J. Biochem. 264:17182 (1989). Opdenakker et al., Virology 171:568, (1989). Using the cDNA of 9-27 gene as a probe possible related sequences have been found in monkeys, rabbits, mice, and cows.

As used herein, a gene member of the interferon gene may, but does not necessarily, contain introns, exons, promoters, operator sequences, termination sequences and the like. The gene may encode an entire functional polypeptide, including whole proteins, such as the 1-8U, 1-8D, 9-27 or 6-16 proteins, or biologically active fragments thereof.

"Interferon-inducible 1-8 polypeptide" is meant to refer to sequences of at least about six amino acids, typically 10 to 25, and up to 100-200 amino acids or more, including up to entire interferon-inducible 1-8 proteins, which have at least some biological activity of the native protein. The nucleotide sequences of members of the interferon-inducible 1-8 gene family are disclosed in Reid et al., Proc. Natl. Acad.Sci. USA 86:840-844 (1989) for 9-27 (GenBank accession no. J004164), and Lewin et al., supra, for 1-8D and 1-8U genes (GenBank accession nos. X57351 and X57352, respectively). The polypeptides will be substantially homologous to an amino acid sequence of human interferon-inducible 1-8 protein. "Substantially homologous" is meant to include sequences which have at least about 65% relatedness, preferably at least 75% homology, and more preferably at least about 85-90% or more homology to the amino acid sequence of a human interferon-inducible 1-8 protein of the invention and still retain at least some biological activity of native protein. Thus, it should be understood that the polypeptide compositions of the present invention need not be identical to any particular interferon-inducible 1-8 protein or amino acid sequence thereof, so long as the subject polypeptides have biological activity.

For example, the polypeptides of interest may be modified by introducing conservative or nonconservative substitutions in the polypeptides, usually fewer than 20 percent, more usually fewer than 10 percent of the amino acids being exchanged. It may be desirable to vary one or more particular amino acids to bind more effectively the RRE or analogous sequence of different retroviruses, for example. As described in more detail below, substitution of a gly-ala dipeptide in the region of amino acids 35-42 of RBP927 (SEQ ID NO:2) enhances inhibition of HIV-1 protein expression.

Therefore, the subject polypeptides may be subject to various changes, such as insertions, deletions and substitutions either conservative or nonconservative where such changes might provide for certain advantages in their

use. "Conservative substitutions" is intended to include, for example, combinations such as gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Usually, the sequence will not differ by more than 20% from the sequence of a 1-8 polypeptide or amino acid subsequence thereof.

In addition, the polypeptide sequence may differ from the natural sequence in the modification of the terminal NH_2 by acylation, e.g., acetylation, or thioglycolic acid amidation, terminal carboxy amidation, e.g., ammonia, methylamine, etc. In some instances, these modifications may provide increased metabolic stability, decreased immunogenicity or the like.

As used herein, "biological activity of a interferon-inducible 1-8 polypeptide" is meant the ability of a polypeptide to inhibit synthesis of at least one viral protein which is essential to viral replication and/or assembly. Offered by way of explanation, and not limitation, for RNA-containing viruses the polypeptide binds to a retroviral RRE element or its analogous sequence and may inhibit the binding or functioning of the Rev protein or its homolog to the mRNA. The inhibition of synthesis refers to suppressing or restraining, in whole or in part. To inhibit protein expression means that synthesis of the translated protein is inhibited in an infected or transfected cell. Inhibition of expression may occur during transcription of the gene encoding the protein, translation of the mRNA encoding the protein or at another step in protein or mRNA synthesis.

Viral infections susceptible to inhibition by the present invention include those RNA- or DNA-containing viruses which are susceptible to inhibition by the interferon-inducible proteins, and more particularly members of the interferon-inducible 1-8 family. Among the RNA-containing viruses are HTLV-I, HTLV-II, HIV-1 (described in U.S. Patent No. 5,037,753 which is incorporated herein by reference), BLV, CAEV (caprine arthritis-encephalitis virus), visna virus and other human or animal retroviruses, including equine infectious anemia virus, particularly those which have RRE

elements or the analogous structure. All lentiviruses and other retroviruses appear to encode functional homologs to Rev, which exert their function by binding to the functional homolog of the RRE RNA target, e.g., the Rex-responsive element (RxRE) of HTLV-I, HTLV-II and BLV. The RRE of the visna virus is described in Tiley and Cullen, J. Virol. 66:3609-3615 (1992), which is incorporated herein by reference. Thus, a retrovirus that produces mRNA having an RRE nucleotide subsequence or functional homolog sequence may be treated by the methods and compositions of the present invention. Among the DNA viruses susceptible to inhibition include those with post-transcriptional mechanisms of gene regulation, such as, e.g., papilloma viruses and possibly adenoviruses.

Pharmaceutical compositions comprising a polypeptide encoded by an interferon-induced 1-8 gene family member, or mutant thereof, and capable of binding to a retroviral RRE and a pharmaceutically acceptable carrier are provided. The compositions are useful in inhibiting retroviral infection in an infected host or cell culture. The compositions act by contacting the polypeptide with retroviral mRNA having an RRE TYPE subsequence. By way of possible explanation but not limitation, the polypeptide binds the RRE segment of the retroviral mRNA and inhibits the up-regulation of expression of the viral protein by Rev. Useful polypeptides for use in the pharmaceutical compositions are RBP927, mutations of RBP927, and 1-8U. Generally RBP927 mutants will have a gly-ala dipeptide substitution in the region of amino acids 35-42 of the wild-type protein.

Polypeptides which bind the RRE segments of retroviral mRNA may be identified by several means. Briefly, in one method cells such as those derived from the U-937 monocyte cell line (ATCC No. CRL 1593) may be used to construct a cDNA expression library as generally described in Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), incorporated herein by reference. The library is screened for

RNA binding proteins with a labeled RRE RNA probe such as RRE330 as described in Constantoulakis et al., Science 259:1314-1318 (1993), which is incorporated by reference herein. Plaques are also screened with a probe such as a probe from the 3' untranslated region of the globin gene to rule out non-specific binding.

Clones which bind the RRE probe but not the non-RRE probe are further screened for specific RRE binding. Mutant RRE probes, such as RREΔ345, RREΔ220, or RREΔ12s as described in Benko et al., supra, may be used to screen the selected clones. Clones which encode polypeptides specific for the wild-type RRE nucleotide sequences are preferred. In addition to RRE region, other functional sites on the viral RNA can be used as probes to identify cellular proteins binding to different areas of HIV-1 and potentially affecting viral expression. Such sites include INS/CRS regions as defined supra.

The cDNA inserts of selected clones are extracted from the phage as a plasmid. The cDNA insert is sequenced by standard techniques such as described in Sambrook et al., supra. Once the cDNA sequence has been obtained, the polypeptide encoded thereby may be expressed.

Another method for identifying RRE-binding polypeptides of the invention is targeted mutagenesis. DNA sequences encoding the polypeptides are mutated by recombination with randomly or site-directed mutated oligonucleotides derived from the native sequence. The mutated genes are then expressed in an interferon-inducible host cell or in a host cell which can express the polypeptide in the absence of interferon induction and then assayed for binding to viral RRE sequences and inhibition of viral protein synthesis.

Mutants which bind viral mRNA or inhibit viral protein synthesis are compared to the native sequence and the specific mutation is identified. The location of the mutation may identify the functional and binding domains of the native proteins. Smaller peptides may be designed which mimic the

binding or functional domains of the native protein. Such functional domains may include regions in the carboxy terminal portion of the RBP927 as described below. The smaller peptides may be synthesized by standard techniques as described herein and screened in a manner similar to the mutated polypeptides.

Polypeptides which are substantially complementary to the amino acid sequences of 1-8 polypeptides (or mutants thereof) or fragments thereof may be produced by a variety of methods. Shorter polypeptides, generally in the range of about six to about fifty amino acids, may be synthesized in solution or on a solid support in accordance with conventional techniques. See, e.g., Stewart and Young, Solid Phase Polypeptide Synthesis, 2nd edition, Pierce Chemical Company, 1984; and Tam, et al., J. Am. Chem. Soc., 105:6442, 1983, both of which are incorporated herein by reference.

Alternatively, hybrid DNA technology may be employed for expression of the desired polypeptide in transformed eukaryotic or prokaryotic host cells, particularly when the polypeptide is a full length 1-8 proteins or substantial portion thereof. Generally, mutated 1-8 proteins will be synthesized by recombinant methods. See, e.g., Sambrook et al., supra.

Procaryotes may be employed for cloning and expressing DNA sequences to produce interferon-inducible 1-8 polypeptides for use in the present invention. Several methods may be employed to produce the desired polypeptides such as those described in Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), incorporated herein by reference.

Several different procaryotic hosts are suitable for cloning the desired DNA sequences. For example, E. coli K12 strain 294 (ATCC No. 31446) is particularly useful. Other microbial strains which may be used include E. coli strains such as E. coli B, and E. coli X1776 (ATCC No. 31537), and E. coli c600 and c600hfl, E. coli W3110 (F^-,λ^-, prototrophic, ATCC No. 27325), bacilli such as Bacillus subtilus, and other

enterobacteriaceae such as Salmonella typhimurium or Serratia marcescens, and various pseudomonas species. When expressed in procaryotes the polypeptides used in the present invention typically contain an N-terminal methionine or a formyl methionine, and are not glycosylated. These examples are, of course, intended to be illustrative rather than limiting.

In general, plasmid vectors containing replication and control sequences which are derived from species compatible with the host cells are used in connection with these hosts. Other vectors, such as λ-phage or cosmids may be employed. The vector ordinarily carries a replication site, as well as sequences which encode proteins that are capable of providing phenotypic selection in transformed cells. For example, E. coli is typically transformed using pBR322, a plasmid derived from an E. coli species. Plasmid pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying and selecting transformed cells. The pBR322 plasmid, or microbial plasmid must also contain, or be modified to contain, promoters which can be used by the microbial organism for an expression of its own proteins. Those promoters most commonly used in recombinant DNA construction include β-lactamase (penicillinase) and lactose promoter systems and a tryptophan (trp) promoter system. While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling a skilled worker to ligate them functionally with plasmid vectors. The promoters are operably linked to an interferon-inducible 1-8 gene family member, or a homolog, mutant or fragment thereof. The promoters may be inducible or constitutive and provide a means to express the encoded interferon-inducible 1-8 polypeptide in the procaryotic host. Following expression, the polypeptide may be purified by standard methods such as described below.

Alternatively, a DNA sequence encoding a 1-8 polypeptide of the present invention may be inserted into a suitable expression vector, which was in turn used to

transfect eukaryotic cells. An expression vector, as used herein, is meant to indicate a DNA construct containing elements which direct the transcription and translation of DNA sequences encoding polypeptides of interest. Such elements include promoters, enhancers and polyadenylation signals. By virtue of the inclusion of these elements operably linked within the DNA constructs, the resulting expression vectors contain the information necessary for expression of the polypeptides of interest.

Host cells for use in expressing recombinant interferon-inducible 1-8 polypeptides of interest (or mutants thereof) include mammalian, avian, insect and fungal cells. Fungal cells, including species of yeast (a.g., Saccharomyces spp., Schizosaccharomyces spp.) or filamentous fungi (e.g., Aspergillus spp., Neurospora spp.) may be used as host cells for producing polypeptides useful in the present invention. Suitable vectors will generally include a selectable marker, which may be one of any number of genes that exhibit a dominant phenotype for which a phenotypic assay exists to enable transformants to be selected. Preferred selectable markers are those that complement host cell auxotrophy, provide antibiotic resistance or enable a cell to utilize specific carbon sources. The expression units may also include a transcriptional terminator. Choice of a particular host and selectable marker is well within the level of ordinary skill in the art.

Cultured mammalian cells may be used as host cells within the present invention. Cultured mammalian cells for use in the present invention may include human monocytoid, lymphocytoid, and fibroblastoid cell lines. A preferred mammalian cell line is the HeLa-tat cells that are HeLa derived cells which produce constitutively HIV-1 Tat (Schwartz et al., supra.

Mammalian expression vectors for use in carrying out the present invention will include a promoter capable of directing the transcription of a cloned gene or cDNA. Preferred promoters include viral promoters and cellular

promoters. Viral promoters include the immediate early cytomegalovirus promoter (Boshart et al., Cell 41:521-530, 1985) and the SV40 promoter (Subramani et al., Mol. Cell. Biol. 1:854-864, 1981). Cellular promoters include the mouse metallothionein-1 promoter (Palmiter et al., U.S. Patent No. 4,579,821), a mouse V_K promoter (Bergman et al., Proc. Natl. Acad. Sci. USA 81:7041-7045, 1983); Grant et al., Nuc. Acids Res. 15:5496, 1987) and a mouse V_H promoter (Loh et al., Cell 33:85-93, 1983).

A particularly preferred promoter is the HIV LTR promoter from HIV-1. Such expression vectors may also contain a set of RNA splice sites located downstream from the promoter and upstream from the DNA sequence encoding the polypeptide or protein of interest. Preferred RNA splice sites may be obtained from adenovirus and/or immunoglobulin genes. Also contained in the expression vectors is a polyadenylation signal located downstream of the coding sequence of interest.

Polyadenylation signals include the early or late polyadenylation signals from SV40 (Kaufman and Sharp, ibid.), the polyadenylation signal from the Adenovirus 5 E1B region and the human growth hormone gene terminator (DeNoto et al., Nuc. Acids Res. 9:3719-3730, 1981). The expression vectors may include a noncoding viral leader sequence, such as the AdenoVirus 2 tripartite leader, located between the promoter and the RNA splice sites. Vectors may also include enhancer sequences, such as the SV40 enhancer and the mouse μ enhancer (Gillies, Cell 33: 717-728, 1983). Expression vectors may also include sequences encoding the adenovirus VA RNAs.

Cloned DNA sequences may be introduced into cultured mammalian cells by, for example, calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981; Graham and Van der Eb, Virology 52:456, 1973). Other techniques for introducing cloned DNA sequences into mammalian cells, such as electroporation (Neumann et al., EMBO J. 1:841-845, 1982), may also be used. In order to identify cells that have integrated the cloned DNA, a selectable marker is generally introduced

into the cells along with the gene or cDNA of interest. Preferred selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker such as the DHFR gene. Selectable markers are reviewed by Thilly (Mammalian Cell Technology, Butterworth Publishers, Stoneham, MA, which is incorporated herein by reference). The choice of selectable markers is well within the level of ordinary skill in the art.

Transfected mammalian cells are allowed to grow for a period of time, typically 1-2 days, to begin expressing the DNA sequence(s) of interest. Drug selection is then applied to select for growth of cells that are expressing the selectable marker in a stable fashion. For cells that have been transfected with an amplifiable selectable marker the drug concentration may be increased in a stepwise manner to select for increased copy number of the cloned sequences, thereby increasing expression levels.

Promoters, terminators and methods for introducing expression vectors encoding interferon-inducible 1-8 polypeptides are well known in the art. Host cells containing DNA constructs of the present invention are then cultured according to standard methods to produce the 1-8 polypeptides. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct.

The interferon inducible 1-8 polypeptides produced according to the present invention, either authentic or recombinant polypeptides or mutant polypeptides, may be purified by a variety of means, including via affinity chromatography, e.g., on an antibody column using antibodies directed against the 1-8 polypeptide. Additional purification may be achieved by conventional chemical purification means, such as liquid chromatography, gradient centrifugation, and gel electrophoresis, among others. Methods of protein

purification are known in the art (see generally, Scopes, R., Protein Purification, Springer-Verlag, NY (1982), which is incorporated herein by reference) and may be applied to the purification of the recombinant 1-8 protein described herein; see also a purification protocol described in U.S. 4,929,604. Substantially pure interferon-inducible 1-8 polypeptide of at least about 50% is preferred, at least about 70-80% more preferred, and 95-99% or more homogeneity most preferred, particularly for pharmaceutical uses. Once purified, partially or to homogeneity, as desired, the 1-8 polypeptide may then be used therapeutically to inhibit retroviral infection in a host.

The purified polypeptides may then be combined in pharmaceutical compositions for a variety of therapeutic uses. The compositions may be administered to persons or animals infected by retroviral pathogens. The polypeptide in the pharmaceutical composition can bind the RRE segment of retroviral mRNA and inhibit binding of the Rev-like proteins to the mRNA. The inhibition of Rev-like protein binding in turn inhibits retroviral protein expression and replication slowing or stopping viral spread to healthy cells.

The compositions also find use for pre- or post-exposure prophylaxis, e.g., HIV prophylaxis following dirty needle injuries to health care workers or routinely accompanying blood transfusions or to persons in danger of becoming exposed to infected body or culture fluids. Retroviruses may not become latent, but merely replicate by a slow and regulated process in the initial phases of infection. Prophylactic treatment of possible exposure may provide a means to prevent development of retroviral disease by inhibiting necessary protein expression in the cells initially infected.

The pharmaceutical compositions are intended for parenteral, topical, oral, or local administration for prophylactic and/or therapeutic treatment. Preferably, the pharmaceutical compositions are administered parenterally, i.e., intravenously, subcutaneously, or intramuscularly.

Thus, this invention provides compositions for parenteral administration which comprise a solution of a interferon-inducible 1-8 polypeptide molecule dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine, and the like, including glycoproteins for enhanced stability, such as albumin, lipoprotein, globulin, etc. These compositions may be sterilized by conventional, well known sterilization techniques. The resulting aqueous solutions may be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such an pH adjusting and buffering agents, tonicity adjusting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc.

The concentration of the interferon-inducible 1-8 polypeptide in these formulations can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. Actual methods for preparing parenterally administrable compounds will be known or apparent to those skilled in the art and are described in detail in, for example, Remington's Pharmaceutical Science, 17th ed., Mack Publishing Company, Easton, PA (1985), which in incorporated herein by reference.

Determination of an effective amount of polypeptide to inhibit viral infection or viral protein expression may be determined through standard empirical methods which are well known in the art. Inhibition of viral protein synthesis and thus efficacy can be monitored with a variety of procedures, e.g., for retroviral proteins the synthesis of retroviral core proteins can be followed in a host, such as the use of p24 or p19 gag antigen capture assays for HIV-1 and HTLV-I,

respectively, which assays are commercially available. An HIV-1 antigen capture assay is also described in U.S. Patent No. 5,104,790, which is incorporated herein by reference.

Compositions of the invention are administered to a host already suffering from an infection, as described above, in an amount sufficient to cure or at least partially arrest the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the severity of the infection or disease and the weight and general state of the patient being treated, but generally range from about 0.5 mg/kg to about 200 mg/kg host body weight of interferon-inducible 1-8 polypeptide per day, with dosages of from about 10 mg/kg to about 100 mg/kg of polypeptide per day being more commonly used. Maintenance dosages over a prolonged period of time may be adjusted as necessary. Generally the desired levels will be approximately 100-1000 times a patient's endogenous levels of interferon-inducible 1-8 polypeptide. It must be kept in mind that the materials of the present invention may be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and general lack of immunogenicity when a human-derived polypeptide is employed to treat human hosts, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these compositions. For veterinary uses higher levels may be administered as necessary while avoiding, however, undesirable toxicities.

In prophylactic applications, compositions containing the interferon-inducible 1-8 polypeptides, e.g., RBP927, mutants thereof, or 1-8U, are administered to a patient susceptible to or otherwise at risk of viral infection to enhance the patient's own antiviral capabilities. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, but generally range from about 0.1

mg/kg to about 100 mg/kg body weight, more commonly from about 1.0 mg/kg to about 50 mg/kg of body weight.

Single or multiple administrations of the compositions can be carried out with the dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of interferon-inducible 1-8 polypeptide of the invention sufficient to effectively inhibit the viral infection in the host's cells.

For the treatment of HIV infection, the pharmaceutical compositions of the present invention may be administered alone or as adjunct therapy. The compositions may be administered with, e.g., AZT or ddI, or as components of vaccines. Administration of the polypeptides of the present invention with transdominant Rev proteins may be especially effective in suppressing HIV protein expression and propagation. Transdominant Rev proteins that inhibit Rev-dependent protein expression are generally described in Mermer et al., Nuc. Acids Res., 18:2037-2044 (1990) and Malim et al., Cell, 58:205-214 (1989), both of which are incorporated herein by reference. Such proteins include RevBL and M10. When administered as adjunct therapy, the compositions of the present invention may be administered in conjunction with the other treatment modalities, or separately at different intervals.

Another aspect of the present invention is the addition of genes encoding expression products of the interferon-inducible 1-8 gene family or homologs or fragments thereof to host cells. Because high copy numbers of viral mRNA can be present in host cells during viral replication, a host cell may not be able to produce sufficient titers of the interferon-inducible 1-8 polypeptides to saturate the RRE segments of viral mRNA. Since the RRE segments are not saturated with interferon-inducible 1-8 polypeptides, the cell may not be able to effectively inhibit viral replication. By increasing the copy number of interferon-inducible 1-8 gene family members or homologs or fragments thereof, higher titers

of interferon-inducible 1-8 polypeptides may be achieved and viral replication may be more effectively blocked.

Addition of the genes to host cells may be accomplished by several methods including those described in International Patent Publication No. WO88/08450, incorporated herein by reference. A particularly useful method is gene complementation as described in Donehower et al., Prog. Med. Virol., 34:1-32 (1987) or McLachlin et al. Prog. Nuc. Acids Res. Mol. Biol., 38:91-135 (1990), both herein incorporated by reference. Briefly, nucleic acid sequences encoding the desired interferon-inducible 1-8 polypeptide may be introduced into retroviral vectors, such as Mo-MLV-based defective expression vectors. The vector may be packaged as virions and used to transfect host cells. Preferably, a retroviral promoter sequence controls the expression of the gene. The gene may also be under the control of a constitutive promoter. Once transfection of the host cells has been achieved, expression of the gene may be accomplished by appropriate means. See Drumm et al., Cell, 62:1227-1233 (1990), Hoeben et al., J. Biol. Chem., 265:7318-7323 (1990), Kasidet al., Proc. Natl. Acad. Sci. USA, 86:8927-8931 (1989), and Morgan et al. AIDS Res. Hum. Retroviruses, 6:183-191 (1990), all of which are incorporated herein by reference.

To enhance delivery of the polypeptides of the invention to virus-infected cells, the polypeptides can be targeted to the cells by a variety of means. For example, the polypeptides can be conjugated to antibodies or binding fragments thereof which bind viral antigens such as gp110 of HIV-1 expressed on the surface of infected cells. The polypeptides can also be incorporated into other targeting vehicles, such as liposomes which comprise an antibody or binding fragment to direct the liposomes to the infected cells. The preparation of immunoliposomes is described in, e.g., U.S. Patent No. 4,957,735 which is incorporated herein by reference.

Interferon-inducible polypeptides of the invention may also find use for the inhibition of retroviral protein

expression in vitro. Cultured cell lines which are infected with retroviruses susceptible to inhibition by the present interferon-inducible 1-8 polypeptides can be treated to inhibit retroviral protein expression and replication. For example, by inhibiting expression of retroviral proteins encoded by RRE containing mRNA, the effect of retroviral proteins which are not encoded by Rev-dependent mRNA on host cells may be determined.

A method of assessing the efficacy of interferon therapy in patients is also provided by the present invention. Detecting changes in the level of interferon-induced 1-8 gene family member expression product, such as mRNA or polypeptide, provides an indication of in vivo interferon activity. Because 1-8 polypeptides can inhibit retroviral protein expression, an increase in the expression level of the interferon-inducible 1-8 genes provides an indication of retroviral protein expression inhibition and hence therapeutic efficacy.

To monitor changes in the level of interferon-induced 1-8 gene family member expression product control values of expression may be determined from cells or other physiological fluids from the general population or from the patient prior to commencement of interferon therapy. Since retroviruses may inhibit endogenous interferon production, determination of each patient's pre-treatment expression products is preferred. The expression product may conveniently be determined by immunoassay of the interferon-inducible 1-8 polypeptide, using polyclonal or monoclonal antibodies prepared according to conventional methods (see, e.g., Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988, which is incorporated by reference herein) using a variety of assay formats. See, e.g., U.S. Patent Nos. 4,642,285; 4,376,110; 4,016,043; 3,879,262; 3,852,157; 3,850,752; 3,839,153; 3,791,932; and Harlow and Lane, supra, each of which is incorporated herein by reference.

Alternatively, expression of mRNA encoding the

interferon-inducible 1-8 family member polypeptide can be monitored in patient cellular samples using a variety of well known techniques, e.g., Northern, dot or slot hybridization using a labeled nucleotide probe. See generally, Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, pp. 7.37-7.57. The level of an expression product of an interferon-induced 1-8 gene family member in cells or physiological fluids of the patient is then determined during therapy. This level is compared to the level of the expression product in cells or physiological fluids not exposed to interferon therapy. Effectiveness of interferon therapy is indicated by an increased level in the measured expression product during therapy.

The following examples are offered by way of illustration, not by way of limitation.

Example I

Isolation of an RRE RNA-Binding Protein

Proteins binding to RRE RNA were identified using a 330-nucleotide uniformly ^{32}P-UTP-labeled RRE RNA probe (RRE330) to screen a λ-ZAP-XR-II cDNA expression library constructed from RNA of the U937 monocyte cell line. E. coli XL-1 blue bacteria were infected with 8.5 x 10^5 plaque-forming units of λ-ZAP XR-II U937 cDNA library (Stratagene) and plated. After 3-4 hours at 42°C, the plates were overlaid with nitrocellulose filters previously soaked in 10 mM IPTG, and incubated for 4-6 hours at 37°C. Duplicate filters from each plate were denatured with 6 M Guanidine-HCl in binding buffer (50 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, 10 mM DTT) for 10 minutes at 4°C. The proteins immobilized on the filters were renatured with sequential lowering of the guanidine-HCl concentration (3 M, 1.5 M, 0.75 M, 0.325 M and 0.187 M for 5 min each); finally, the filters were

equilibrated with binding buffer alone. The filters were incubated in blocking solution (1x Denhardt's, 50 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, 10 mM DTT) at room temperature for one hour, rinsed in binding buffer and then hybridized with 0.5-1.0 x 10^6 cpm/ml of [^{32}P]-UTP-labeled RRE or globin RNAs in the presence of 0.1 mg/ml tRNA for one hour. After extensive washing, the filters were exposed to X-ray film and plaques that scored positive with the RRE and negative with the globin probe were identified and picked individually. After four rounds of plaque purification using RRE and globin RNA probes, 12 clones were isolated and plated individually on a E. coli lawn. After IPTG induction the nitrocellulose lifts were incubated with labeled RRE330, RREΔ345, RREΔ12s, TAR or globin RNA probes. RRE probes were described previously, Benko, et al., New Biol., 2:1111-1122 (1990). The globin probe comprises a 320-nucleotide RNA spanning the StyI to PstI fragment from the 3' untranslated region of the human β-globin gene (Genebank HUHMBB, nt:63862-64182). The TAR RNA comprises nucleotide +1 to +180 and was transcribed from pLG1.

To determine whether the clones expressed human proteins which bound specifically to the RRE probe, the binding of the cloned proteins to different mutated forms of RRE was assessed. In addition to RRE330, RRE mutants RREΔ345, RREΔ12s, and RRE220 were tested. RREΔ12s lacks 67 nucleotides comprising hairpin loops 1 and 2, which contain the Rev binding site, does not bind Rev in vitro, and does not function in vivo. RREΔ345 lacks 82 nucleotides of the RRE comprising hairpin loops 3, 4, and 5, and RRE220 has part of the long stem deleted. RREΔ345 and RRE220 have been shown to bind Rev and to function in vivo, Benko, et al., supra. A radiolabeled RNA containing the Tat-binding site (TAR) of HIV-1 was employed as an additional RNA sample known to form a strong secondary structure. The extent of binding of the probes by lysates of the 12 clones was determined after incubation with the RNA probes which were radiolabeled to a similar specific activity. One clone (clone # 11) bound to the intact RRE (RRE330), the RRE220, and RREΔ12s, but failed

to bind to RREΔ345 and to TAR RNA (Fig. 1A). This suggested that the recombinant protein produced by clone #11 specifically bound to the region containing hairpin loops 3, 4, and 5 of the RRE.

EXAMPLE II

Sequence of the β-gal-RBP927 Protein

The 850-nucleotide cDNA insert of clone #11 was excised as a plasmid from the phage together with part of the vector and was completely sequenced according to the general protocol of the Sanger Dideoxy-mediated chain-termination method, described in Sambrook et al., Molecular Cloning A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

The cDNA insert contained an open reading frame of 97 amino acids, positioned in frame with β-galactosidase in λ-ZAP. The first 27 amino acids of RBP927 (underlined in Fig. 1B) were replaced by the 37 amino-terminal amino acids of the lacZ gene. The sequence of the insert begins with Ser_{28} and consists of the 118 carboxy-terminal amino acids of the 9-27 protein (Genebank accession number: J04164).

A computer search of the Genebank database revealed identity with the human interferon-inducible gene 9-27 (entry J04164), Reid., et al., Proc. Natl. Acad. Sci. USA, 86:840-4 (1989). The 9-27 gene encodes 125 amino acids. The cDNA insert in clone #11 comprises the 97 amino acids of the carboxyl terminus of the 9-27 protein, starting at Ser_{28}. The protein was subsequently designated "RNA binding protein 927" or RBP927.

EXAMPLE III

Expression of RBP927 in Bacteria and Purification

The gene encoding protein RBP927 was cloned and expressed in bacteria as a fusion protein with glutathione transferase (GST). A cDNA fragment of 555 bp was generated after reverse transcription of RNA from interferon-induced HeLa cells, followed by polymerase chain reaction (PCR)-amplification. Total RNA was isolated from HeLa cells after induction with IFN-α for 48 hours and reverse transcribed using a mix of random hexamer primers, Ciminale, et al., J. Virol., 66:1737-1745 (1992), followed by PCR amplification with gene 9-27 specific primers. The primers used were: 5'-CCCCCGGGCCAGAAGATGCACAAG-3" (sense) (SEQ ID NO: 4) and 5'-TCACAAGCACGTGCACTTTATTGAA-3' (antisense) (SEQ ID NO: 5). ATG initiation codon and polyadenylation signal of 9-27 are underlined. The amplified 555 bp fragment was digested with SmaI digested pGEX-2T vector (Pharmacia/LKB). The resulting construct (pGST927) was electroporated in E. coli (JM109). 9-27 was purified as GST927 fusion protein or after cleavage with thrombin which resulted in a protein containing six additional amino acids at the amino terminus as detailed in Fig. 1C.

This cDNA fragment was then subcloned into the bacterial expression vector GEX-2T, (Smith et al., Gene 67:31-40 (1988). 9-27 was produced as a fusion protein with glutathione S-transferase. After IPTG induction the GST-927 protein was purified by binding to glutathione-agarose beads. 9-27 was purified after cleavage by thrombin and contains 6 additional amino acids at the amino terminus (Fig. 1C).

EXAMPLE IV

Characterization of the Specificity for RRE

The binding affinities of bacterially produced 9-27 protein to RRE330 and RREΔ345 were compared using an in vitro filter binding assay. An overnight culture of GST-927 was diluted 1/100 into fresh LB medium and grown to OD550 of 0.7. The production of the fusion protein was induced by addition of IPTG (10mM final) and the culture was grown for an additional 2 hours. The cells were pelleted and resuspended in buffer A (50 mM Tris-HCl pH 7.5, 1 mM EDTA pH 8.0, 5 mM DTT, 50 mg/ml PMSF). After freezing for 2 minutes in liquid nitrogen the cells were lysed by sonication on ice. TritonX-100 and NaCl were added to a final concentration of 1% and 3M, respectively, and the lysate was incubated for 30 minutes at 4°C on a rotating wheel. The cellular extract was pelleted at 12,000 rpm for 15 minutes and the supernatant was dialyzed extensively (4 x 500 ml) against buffer A containing 150 mM NaCl at 4°C. The dialysed sample was loaded on a glutathione-sepharose affinity column equilibrated with buffer A followed by several washes. To cleave 9-27 protein from the GST portion, the column was washed extensively with buffer A containing 2.5 mM $CaCl_2$ and no EDTA and no PMSF. Subsequently, the sepharose beads were incubated with 20 μg human thrombin in the same buffer for 30 minutes at room temperature and washed 5 times with 1 ml of buffer A. The positive fractions were dialysed extensively against storage buffer (20 mM Tris-Cl (pH 8.0), 0.2 mM EDTA (pH 8.0), 150 mM NaCl, 5 mM DTT, 50 μg/ml PMSF and 20% glycerol), concentrated 10 times with Centricon 10, and stored at -70°C.

RNA-protein complexes were measured by filter-binding. 40 pM of the thrombin-cleaved RBP927 was incubated with 0.1 pM of [^{32}P]-labeled RRE330 RNA or RREΔ345 RNA in the presence of increasing amounts of unlabeled competing E. coli tRNA (Boehringer) in 50 μl binding buffer (10 mM Tris pH 7.5, 1 mM EDTA, 50 mM NaCl, 2 mM DTT, 0.01% BSA, 20 units/ml RNAsin) for 20 minutes at room temperature and then stored on

ice until the filtration assay was performed. Nitrocellulose filters (Schleicher and Schuell HA, 25 mm diameter, 0.45 μm pore size) were mounted on standard filtration apparatus and washed first with water and then with binding buffer. The samples were spotted on the filters and allowed to be absorbed under vacuum. The filters were then washed extensively with binding buffer, air dried for 30 minutes and the bound radioactivity was measured in a scintillation counter. The values shown in Fig. 2A represent averages of triplicate determinations. The radioactivity bound in the absence of competing RNA was taken as 100%. Fifty percent inhibition of binding to RREΔ345 was reached with 20 ng/ml tRNA, while approximately 10,000-fold more tRNA was required produce 50% inhibition of binding to RRE330.

The specificity of binding of RBP927 to RRE330 RNA was also determined. The extent of binding was determined in the presence of increasing amounts of various RNA and DNA competitors such as intact RRE330, RREΔ345, tRNA, globin RNA, and single- and double-stranded DNA (Fig. 2B).

RBP927 was bound to labeled RRE330 RNA as described above in the presence of increasing amounts of unlabeled competitor nucleic acids: RRE330 RNA, RREΔ345 RNA, globin RNA, E. coli tRNA, single-stranded DNA (sheared Salmon sperm DNA, Sigma Chem. Co., boiled and rapidly cooled) or doubled-stranded DNA (sheared Salmon sperm DNA, Sigma Chem. Co., boiled and allowed to cool gradually. RRE330 competed very efficiently for complex formation, while a 10^4 to 10^5 fold excess of RREΔ345 or tRNA, respectively, was required to reach 50% inhibition of complex formation. None of the DNA preparations competed for binding. These data demonstrate that the binding of RBP927 protein competed best with RRE330 RNA, while it competed poorly with the other RNAs or not at all with the DNAs. These experiments further demonstrated that 9-27 bound to RNA but not to DNA, and that the binding for RRE was specific.

EXAMPLE V

Recombinant Expression of RBP927 in Human Cells

RBP927 was recombinantly produced in human cells. The 9-27 cDNA was PCR-amplified and subcloned into a eukaryotic expression vector, pLDKpA, Mermer, et al., Nucleic Acids Res., 18:2037-2044 (1990), between the HIV-1 long terminal repeat (LTR) promoter and the SV40 polyadenylation site, generating a plasmid identified as pL927.

Total RNA was isolated from HeLa cells after induction with IFN-α and reverse transcribed and PCR-amplified as described above, specifically the 9-27 cDNA. The primers used were: 5'-TTCCCCAAAGCCAGAAGATGCA-3' (sense) (SEQ ID NO: 6) and 5'-TCACAAGCACGTGCACTTTATTGAA-3' (antisense) (SEQ ID NO: 7) generating a 553-bp of 9-27 cDNA; 5'-CATGAACCACATTGTGCAAACCT-3' (sense) (SEQ ID NO: 8) and 5'-GAAACATATACACTTTATTGAATG-3' (antisense) (SEQ ID NO: 9) generating a 626-bp 1-8D cDNA; 5'-CATGAGTCACACTGTCCAAACCT-3' (sense) (SEQ ID NO: 10) and 5'-CCAGAAACACGTGCACTTTAT-3' (antisense) (SEQ ID NO: 11) generating a 563-bp 1-8U cDNA. Initiation codons and polyadenylation signals are underlined. The PCR-fragments were blunted with Klenow fragments of the DNA polymerase and ligated into SmaI-digested pBluescript KS II (Stratagene). The cDNAs were ligated as HindIII-BamHI fragments into the HindIII/BamHI double-digested pLDKpA eukaryotic expression vector, Mermer et al., supra, resulting in pL927, pL18U and pL18D. pLdKpA contains the HIV-1 LTR promoter, a polylinker and the Simian virus 40 polyadenylation signal (pA).

The production of RBP927 in human cells was verified by transfection experiments followed by immunoprecipitation of the protein by a rabbit antibody raised against the purified, bacterially synthesized GST-RBP927 fusion protein. HLtat cells were transfected with 10 μg pL927. HLtat cells are produced from a HeLa-derived cell line that produces constitutively HIV-1 Tat, Schwartz, et al., submitted for publication, which is necessary for the expression of pL927. As a control, untransfected HLtat cells were cultivated with

(+) or without (-) 500 IU/ml of IFN-α for 48 hours. The cells were labeled with ^{3}H-leucine for 5 hours and extracts were immunoprecipitated using a 1:50 dilution of an anti-927 rabbit antisera. Anti-927 antisera was generated against the GST-927 fusion protein as follows. GST-RBP927 was eluted from the glutathione-sepharose affinity column with buffer A containing 10 mM glutathione. The protein was purified from a 12.5% SDS polyacrylamide gel after visualization with 0.1M KCl. The gel piece was minced in PBS and injected into rabbits. See Fig. 3A.

The cellular location of RBP927 was determined by immunofluorescence experiments using FITC-labeled anti-rabbit antibody. HLtat cells were transfected with pL927. One day posttransfection, the cells were fixed and analyzed by indirect immunofluorescence as described (FELBER et al. Science 229:657-679 (1985)) using a 1:50 dilution of the RBP927 antisera followed by FITC-coupled secondary rabbit antibody. The 927 protein was localized primarily around the nucleus and in the Golgi region of the transfected cells. See Fig. 3B.

EXAMPLE VI

Inhibition of Proviral Protein Expression by RBP927

The in vivo effect of RBP927 on HIV-1 protein expression was analyzed to determine the significance of RBP927 binding to RREs. Provirus clone pHXB2 was transfected with increasing amounts of the RBP927 producing plasmid pL927 in HLtat cells and the production of proteins and RNA measured. HLtat cells were transfected with 5 μg of the molecular clone pHXB2 (FISHER et al., Nature 316:262 (1985)), 2 μg of luciferase expression plasmid (pRSVluc) (DeWit, J. Mol. Cell. Biol. 7:725 (1987)) and 0, 1, 5, and 10 μg of pL927. The total amount of DNA in the transfection mixture was adjusted to 17 μg using carrier DNA. One day later, the cells were harvested for

protein and RNA analysis. Proteins expressed by the transfected cells were analyzed by Western blot analysis. The proteins were separated on 12.5% SDS/PAGE gels, electrotransferred onto nitrocellulose filters and probed with HIV-1 patient serum followed by ^{125}I-coupled protein A, as described in Hadzopoulou-Cladaras et al., J. Virol. 63:1265-1274 (1989). As demonstrated in Fig. 4A, a dramatic decrease in Env and Gag protein production occurred in a dose-dependent fashion. The decrease in Gag production was quantitated using a p24gag antigen-capture assay. The expression of the cotransfected luciferase producing plasmid (pRSVluc) was only marginally affected by RBP927. See Fig. 4B.

To determine the mechanism of this inhibition, the levels of viral mRNAs were analyzed. In parallel experiments total RNA was extracted from cells transfected as described above. Total RNA was isolated as previously described (Krawczyk et al., Biochem. 165:20 (1987)). After transfer, the blots were hybridized with ^{32}P-labeled DNA probe from the HIV-LTR region, Schwartz et al., supra, that detects the unspliced (U), intermediate spliced (I) and small, multiple spliced (S) viral mRNA species. The blot was subsequently washed and rehybridized with the 9-27 and human β-actin cDNA specific probes (D'Agostino et al., Mol. Cell. Biol. 12:1375 (1992)). Luciferase production from the cotransfected pRSVluc was measured as a internal control of transfection efficiencies (Solomin et al., J. Viol. 64:6010 (1990)). Hybridization of Northern blots with an HIV-1 LTR probe that detects all three classes of HIV-1 RNAs revealed a dramatic reduction of the levels of unspliced and intermediate-spliced mRNAs in the presence of pL927. The presence of RBP927 had no detectable effect on the levels of the small, multiple spliced viral RNAs or the cellular β-actin RNA. Rehybridization of the blot with a RBP927-specific probe confirmed the presence of increasing amounts of RBP927 RNA. See Fig. 4C. The expression of RSV-luciferase was measured as a control of transfection efficiencies.

The inhibition of expression of viral structural proteins was also observed using subgenomic gag expression plasmid. Protein expression from plasmid pCgagA2 that contains the HIV-1 gag gene flanked by the LTRs was analyzed after cotransfection with a Rev-producing plasmid (pL3srev) and a 1-, 5-, 10- and 20-fold excess of pL927. Western blot analysis of proteins extracted from cells transfected with 1 μg of gag expressing plasmid pCgagA2, Felber et al., supra, 0.5 μg of the Rev expressing plasmid pL3crev, Felber et al., supra, and increasing amounts of pL927 were performed. All transfections were done in the presence of the luciferase expression plasmid pRSVluc. The presence of increasing amounts of RBP927 produced to a drastic decrease in Gag production (Fig. 5A).

Quantitation of this data using the $p24^{gag}$ antigen capture assay demonstrated that gag production is inhibited to about 50% in the presence of equimolar amounts of Rev- and RBP927-expressing plasmids. Expression of the cotransfected luciferase expressing plasmid is only marginally affected. See Fig. 5B.

EXAMPLE VII

RBP927 does not Interfere with LTR Promoter

To demonstrate that RBP927 did not inhibit the expression of any LTR driven gene through interfering with HIV promoter activity, pL927 was cotransfected with p37M1234 which contains $p37^{gag}$ gene of HIV-1 having several point mutations in its coding sequence that alleviate the inhibitory effects of RBP927 as described, Schwartz et al., supra. The expression of p37M1234, which lacks RRE sequences, is Rev independent.

pCgagA2 requires Rev for gag expression, Felber et al., supra. p37M1234 contains several silent point mutations in the gag coding region resulting in high levels of Rev-

independent Gag expression, Schwartz et al., supra. Both transfection mixtures contained the Rev expression plasmid pL3crev. Gag production was quantitated using the antigen-capture assay. As shown in Figure 6A, there is no significant inhibition of Gag expression even in the presence of 10 μg of pL927 that abolishes gag expression from pCgagA2.

Using similar methods, data was obtained upon cotransfection with pNLp19, Schwartz et al., supra, which expresses the $p19^{gag}$ gene of the Rous Sarcoma Virus (RSV) linked to the HIV-1 LTR promoter. HLtat cells were transfected with 5 μg pNLp19 and increasing amounts of pL927. One day later, the cells were harvested for RNA and protein analysis. Total RNA was isolated and Northern blots were hybridized as described above. The RSV probe was described previously, Schwartz et al., supra. Western blot analysis was performed using p19gag antisera. Expression of p19gag was not affected by the presence of Rev in trans (Schwartz et al., supra). See Fig. 6B. These data indicate that RBP927 does not act by inhibiting LTR-driven genes through promoter interference.

EXAMPLE VIII

Inhibition by RBP927 Affects only Rev-dependent Gene Expression

To study whether RBP927 inhibits only Rev-dependent gene expression and not all RRE-containing RNAs, expression of a Rev-independent gene containing RRE was observed in the presence of RBP927. A plasmid containing the RSV $p19^{gag}$ gene linked to the Cytomegalovirus (CMV) immediate early promoter was constructed (pCMVp19R). HLtat cells were transfected with 5 μg pCMVp19R and increasing amounts of pL927 as indicated. One day later, the cells were harvested for RNA and protein analysis. Total RNA was isolated and Northern blots were hybridized as described above. The RSV probe was described

previously, Schwartz et al., supra. Western blot analysis was performed using anti-p19^{gag} antisera. Expression of p19^{gag} was not affected by the presence of RRE in cis and Rev in trans (Schwartz et al., supra.). Cotransfection of increasing amounts of pL927 did not affect RNA (Fig. 6C) and protein expression (Fig. 6D).

EXAMPLE IX

Provirus Inhibition by Other 1-8 Proteins

Cotransfection experiments using two other members of the interferon-induced 1-8 gene family, 1-8U and 1-8D, were performed after cloning the coding regions of the two genes in the same expression vector as described for RBP927. The function of the genes was assayed in cotransfection experiments similar to those described for RBP927. Two sets of HLtat cells were transfected with 2 μg of the HIV-1 proviral clone pHXB2 and 1 μg of the gag expression plasmid pCgagA2, respectively, in the presence of increasing amounts of pL927, pL18U, and pL18D. All transfection mixtures contained 1μg of pRSVluc and were adjusted to a total of 17 μg DNA. The transfection mixture containing pCgagA2 also contained 0.5 μg of the Rev-expressing plasmid Pl3crev. Gag expression was quantitated by the HIV-1 p24^{gag} antigen-capture assay.

As compared to RBP927, the presence of increasing amounts of 1-8U gene encoded polypeptide produced a limited inhibitory effect on HIV-1 protein expression in cells cotransfected with the proviral clone HXB2 or the gag expression plasmid pCgagA2. Cotransfection of these cells with the 1-8D expressing plasmid did not inhibit HIV-1 protein expression (Figs. 7A and 7B).

EXAMPLE X

RBP927 Inhibition on HTLV-I Protein Expression

The effect of RBP927 on the expression of HTLV-I RRE containing genes was analyzed to determine whether RBP927 possesses antiviral activity against other retroviruses. Two μg of CS-HTLV-I (a full length molecular clone that produces all viral proteins and viral particles, Ciminale, et al., J. Virol., 66:1737-1745 (1992)) and increasing amounts of RBP927 expressing plasmid pL927 were transfected into HLtat cells. Two days later, the cells were harvested and analyzed for gag expression using the HTLV-I $p24^{gag}$ antigen-capture assay.

In one experiment 5 μg of the CS-HTLV-1 plasmid were cotransfected with 1 μg of RSVLuc (as above) and increasing amount of pL927 (0.5, 1.0, 5.0 and 10 μg). In this experiment there was a 55% inhibition of HTLV-1 p24gag antigen in 0.5 μg pL927 concentration and a 90% inhibition in >1.0 μg concentrations whereas there was no significant inhibition of the Luciferase activity. In two subsequent experiments 2 μg of the CS-HTLV-1 plasmid were cotransfected with 1 μg of RSVLuc (as above) and increasing amount of pL927 (0.5, 1.0, 5.0, and 10 μg). See Fig. 8.

These results demonstrate that RBP927 inhibits HTLV-I expression. Additional experiments using a gag expression plasmid containing the Rex responsive element (RXRE) of HTLV-I (pCgagRXRE, Benko et al., supra) were performed to determine whether RBP927 inhibits the function of Rex. In three independent experiments 2 μg of pCgagRxRE was cotransfected in HeLa-tat cells with 0.5 μg pL3Rex (Benko et al., supra.) that provides the Rex protein in-trans, 1 μg of pRSVLuc (for internal control) and increasing concentrations of pL927 plasmid (0.5, 1.0, 5.0 and 10 μg). This indicates that RBP927 inhibits the function of the Rex protein in HTLV-I. The effects of the proteins of the 1-8 family on the expression of other retroviruses were studied and the results were that CAEV and Bovine Leukemia virus (BLV) are inhibited by RBP927 and RBP18U.

EXAMPLE XI

Synergistic HIV-1 Inhibition Between RBP927 and RevBL

This example demonstrates the synergistic inhibition of HIV-1 expression in transfected cells by RBP927 and transdominant Rev proteins. Expression of $p24^{gag}$ by these cells was markedly inhibited even in cells that expressed RBP927 and transdominant Rev at low levels.

Plasmids pL927 and pLRevBL were introduced singly or together into cells transfected with the infectious molecular HIV-1 clone, pNL43 (Adachi et al., J. Virol., 59:284-291 (1986)). As demonstrated in Fig. 9, cotransfection of cells with an infectious molecular clone pNL43 of HIV-1 and 1 μg of either pL927 or pLRevBL decreased expression of $p24^{gag}$ by pNL43. The level of inhibition was as high as 80% as compared to control. Similar levels of $p24^{gag}$ expression inhibition were obtained by transfecting cells with infectious molecular clone pNL43 of HIV0-1 and 0.1 μg of each of pL927 and pLRevBL. This demonstrates that the two Rev inhibitors downregulate Rev-dependent HIV-1 expression in a synergistic fashion. The synergistic inhibition was prominent at low levels of $p24^{gag}$ expression.

Infected cells were then transfected with 0.1 μg of one plasmid and increasing amounts of the other. Fig. 10 illustrates that addition of as little as 0.01 μg of pL927 to cells transfected with 0.1 μg of pLRevBL resulted in significantly increased inhibition of $p24^{gag}$ expression as compared to the level of inhibition produced by transfection of 0.01 μg of pL927 alone. Fig. 11 illustrates similar effects when increasing concentrations of pLRevBL are added to infected cells transfected with 0.1 μg of pL927. The observed reaction kinetics differed, however. At least 0.1 μg of pLRevBL was required to significantly inhibit $p24^{gag}$ expression in cells transfected with 0.1 μg of pL927. This indicates that addition of even small amounts of RBP927 to infected cells expressing RevBL may dramatically increase inhibition of HIV-1 expression.

EXAMPLE XII

Effects of Coexpression of Different 1-8 Family Members on HIV-1 Protein Expression

This example demonstrates the effect of cotransfection of plasmids containing different members of the interferon-inducible 1-8 gene family. Additive inhibition of HIV-1 protein expression was observed with co-transfection RBP927 and 1-8U. Cotransfection with RBP927 and 1-8D resulted in partial abrogation of the inhibitory effect of RBP927.

HeLa-tat cells were transfected with HIV-1 proviral clone pHXB2 with pCMVCAT a plasmid containing the human cytomegalovirus early promoter driving the CAT gene and increasing amounts of pL927, pL18U, or both. Fig. 12A illustrates that cotransfection of pL927 and pL18U provided additive inhibition of viral protein expression. Transfection of cells with 1 μg of pL927 or pL18U alone provided inhibition of 35-40% compared to control while the same level of inhibition was achieved by cotransfection of 0.5 μg of each plasmid.

HeLa-tat cells transfected with pHXB2 and pCMVCAT as above were cotransfected with pL927 and pL18D. Fig. 12B demonstrates that viral protein inhibition by RBP927 was abrogated by the addition of 1-8D.

EXAMPLE XIII

Inhibition of HIV-1 Protein Expression by RBP927 Mutants

This example demonstrates HIV-1 protein expression by several RBP927 mutants. Functional domains were identified that effect the inhibitory effect of RBP927. Mutants possessing increased inhibition of viral protein expression were identified.

Functional sites in RBP927 were mapped by producing frame shift, deletion, and point mutants in plasmid pL927. The mutations were verified by DNA sequencing. Protein expression inhibition was assayed by cotransfection of mutant pL927 plasmids with pNL43 as described above and compared to inhibition by wild-type RBP927.

Fig. 13 illustrates the RBP927 frame shift and deletion mutants assayed. Wild-type RBP927 is on the top line. FS-StyI is a frame shift mutation at the StyI position of wild-type RBP927 containing 20 N-terminal amino acids of RBP927 and an additional 44 new amino acids ending at a termination codon produced by the frame shift. FS-Tth111I is a frameshift mutation at the Tth111I of wild-type RBP927 containing 35 N-terminal amino acids of RBP927 and an additional 28 new amino acids. Del-ApaI has a 14 amino acid deletion (amino acids 76-89 of SEQ ID NO:2) in RBP927. Del+FS-ApaI has the same deletion and 81 new amino acids following the deletion caused by a frame shift.

Immunoprecipitation assays with polyclonal antibodies raised against bacterially produced RBP927 showed comparable levels of expression of RBP927 and the mutants in transfected HLtat cells. Immunofluorescence demonstrated perinuclear localization of wild-type RBP927. Of the above mutants, only Del-ApaI demonstrated perinuclear localization. Also, only Del-ApaI inhibited Rev-dependent HIV-1 protein expression in cotransfection experiments (See Fig. 13). This indicates that an essential functional site exists in the carboxy terminal region of RBP927.

Point mutations were introduced by site-directed mutagenesis. Fig. 14 illustrates the amino acid alterations resulting from mutations in the coding region. The mutations substituted two amino acids with gly-ala. Functional analysis of the mutants demonstrated that the mutant containing the mutation at amino acids 62/63 of SEQ ID NO:2 was inactive in cotransfection experiments with HIV-1 molecular clones. This identifies a necessary functional domain of RBP927. This region is positively charged and conserved though all three described interferon inducible 1-8 gene family members and likely represents the RNA binding site. Other mutations clustering in the region of amino acids 35-42 of SEQ ID NO:2 were demonstrated to enhance inhibition of HIV-1 protein expression.

EXAMPLE XIV

Levels of RBP927 mRNA in HIV Infected Individuals

This example demonstrates the level of RBP927 mRNA in peripheral blood mononuclear cells (PBMCs) of persons infected by HIV-1 during all phases of infection. Oligonucleotide probes to RBP927 mRNA were prepared and used in RNA polymerase chain reaction of PBMCs of healthy and HIV-1 infected persons. Fig. 15 demonstrates a increased expression of RBP927 in PBMCs of HIV-1 infected individuals. This is evidence of persistent partial induction of the interferon system during all phases of HIV-1 infection.

RNA Isolation: Peripheral blood mononuclear cells (PBMCs) were isolated from HIV-1 infected human blood by Histopaque (Sigma) gradients. 5 x 10^6 cells were lysed in 1 ml of RNazol (TELTEST) and the RNA was extracted as described by the manufacturer (0.1ml of chloroform was added to the homogenate, then briefly vortexed and incubated on ice for 15 minutes. The lysate was centrifuged at 15,000 rpms for 15 minutes, the upper aquous phase was transferred to a new tube and an equal volume of isopropanol was added. The sample was stored at -20°C for 45 minutes and centrifuged as described above. The pellet was rinsed in 70% ethanol and air-dried.) The RNA pellet was resuspended in 100μl of water.

Reverse transcription: The total number of PCR reactions needed for an experiment was determined and reverse transcription of a sufficient quantity of RNA for all PCR reactions was performed. The standard reverse transcriptase reaction for one PCR reaction is described below, and multiplied by the number of PCR reactions required: 2μl of total RNA was added to 5μl 10X PCR reaction buffer (PEC), 5μl dNTP mix (1.25mM each DNTP), 2.5μl of 1mg/ml pd(N)6 (Pharmacia), 10 units of RNAsin (Promega),5 units of AMV reverse transcriptase (Boeringer Mannhein) and the volume was brought to 50μl. The reaction was incubated at 45°C for 2 hours.

Kinasing the 5 specific oligonucleotides: The oligonucleotides used are described in Figures 1 and 2. 5'

specific oligonucleotide for each class of HIV-1 specific transcript (25pMol/100μl PCR reaction) was kinased using T4 polynucleotide kinase (NEG) in buffer supplied by the manufacturer. A 2μl aliquot was removed and the total number of counts determined by scintillation counter. For analysis of patient samples, the kinased oligo contained about 300,000 counts/μl.

cDNA markers: For measurement of the 1-8 gene family expression the following combinations of primers were used:

Detection/ Name (Location)	Sequence	TM (^{0}C)
Specific 927		
7796(125-146)927 sense	TTCCCCAAAGCCAGAAGATGCA	66.9
10842(474-453)927 anti-sense	ACTGTCACAGAGCCGAATACC	56.6
Non-speicifc 927		
7796(125-146)927 sense	TTCCCCAAAGCCAGAAGATGCA	66.9
10315(455-436)927 anti-sense	CCAGTGACAGGATGAATCC	58.3
1-8D		
8277(278-301)1-8D sense	CATGAACCACATTGTGCAAACCT	59.1
10579(691-673)1-8D anti-sense	GATGCCTCCTGATCTATCG	58.6
1-8U		
8276(236-259)1-8U sense	CATGAGTCACACTGTCCAAACCT	54
10578(652-631)1-8U anti-sense	GATGCCTCCTGATCTATCCA	60.9

A collection of cDNA clones encoding most HIV-1-specific transcripts produced during an infection of cultured cells by HIV-1 strain HXB-2 were assembled. Mixtures of these plasmids encoding the unspliced, multiply-spliced and singly-spliced transcripts was PCR-amplified in parallel to the reverse transcribed samples of interest. These markers served a dual purpose. 1) they allow the visualization of each individual transcript; 2) several concentration of the marker mix was amplified that provide a standard curve from which to estimate the amount of HIV-1 specific mRNA present in the original sample.

The marker mixes were made by first diluting all plasmids to a concentration of 1.0 pg/μl in water in the presence of 10ng/μl Bluescript (Bluescript added for DNA

stability). An equal amount of each of the plasmids encoding the multiply-spliced transcripts, at 1.0pg/μl, were assembled for the multiply spliced marker mixture. For the singly spliced mix, 100 μl of each of 4 plasmids were placed in a 1 ml final volume of Bluescript (10ng/μl) containing water. The unspliced marker was simply a 1:10 dilution of the HXB2 plasmid in 10ng/μl Bluescript-H20, as were markers for the cellular genes such as actin and 9-27. Aliquots of these mixes were frozen on dry ice, kept at -80°C and thawed a single time before use. The markers were used directly for 20 cycles PCR when examining HIV-1 expression in tissue culture, however were further diluted for use with 30 cycle PCR. The HIV-1 specific cDNA mixes, and 9-27 marker were diluted 1;1,000 and 1;10,000. 10-0.1fg are amplified from these dilutions. The actin marker plasmid was not as efficiently amplified, the plasmid was therefore diluted so that markers between 50 and 5 fg were amplified.

PCR Reaction. For each 100μl PCR reaction: 50μl of the RT reaction or marker mix in 50μl 1x PCR buffer (PEC) was added to 50μl of the PCR "master mix". The master mix was assembled for each series of samples amplified with the same oligonucleotide pair. The mixture contained a multiple of the following reagents depending on the number of samples to be analyzed: 5μl 10x reaction buffer (PEC), 5μl 1.25mM dNTPs, 25 pmoles of each oligo and 0.2μl Amplitaq (PEC, 2.5U/μl), brought to a total volume of 50 μl. The HIV-1 specific PCR reactions using the 5' primer located immediately before the major splice donor were processed in parallel. A negative control lacking reverse transcriptase was included for the gag reactions to test for DNA contamination, such a control was not necessary for the spliced transcripts.

A B-actin-specific reaction was amplified in parallel as an internal control for each sample.

Reactions containing a reagent control and 3 dilutions (see above) of the cDNA marker mixes were used as negative and positive controls for each experiment. Detection of actin in a linear range for these samples required the

dilution of each reverse transcription reaction (originally containing 2μl/100μl RNA) 10,000 or 100,000 fold before amplification. Similarly, detection of 9-27 required a 1000X dilution of the RT reactions.

In order to decrease the chance of contamination of the reactions the reverse transcription, kinasing and assembly of the PCR reactions was performed in a PCR clean room. The master mix was placed in the labelled 500μl thin walled tubes (PEC, cat. no.) and the RT reactions were added bringing the final volume to 100μl in each tube. The tubes containing the master mixes to be used for the marker amplification were brought to the main lab and the marker DNA was added.

The PCR reactions were performed in the Gene-Amp9600 (PEC) thermocycler when the temperature was 80°C. The PCR reaction consisted of 30 cycles, each cycle included a 45 second denaturation step at 94°C with an additional 30 second hold at this temperature for the first cycle, followed by 1 min at 60°C and 2 minute at 72°C. Following the amplification cycles was a 7 minute final extension at 72°C.

Polyacrylamide gels: The products were separated using denaturing polyacrylamide gel electrophoresis using the 1070 model V16 (BRL) vertical gel apparatus. The multiply-spliced transcripts were run on 6% gels, all others were run on 4% gels. The gel mixtures contained instapage acrylamide mix to the appropriate percentage (7.5ml for 6% gels and 5ml for 4% gels) added to 5ml of 20X TBE and 25g urea (50%) the volume brought to 50 ml with water. The acrylamide mixes were dissolved, degassed, filtered through a 0.4 μM filtration unit (Millipore). 500μl APS and 25 μl TEMED were added mixed well and the gel was poured and hardened for 20 minutes. The gels were pre-run at 350V for 30 minutes.

Sample preparation for loading. 5 μl (5μl/100μl reaction volume) of the sample was added directly to 15 μl of formamide containing stop mix (USB) or the entire sample was dryed down using a speed vacuum and then re-dissolved in the same volume of stop mix (USB) or the entire sample was dryed down using a speed vacuum and then re-dissolved in the same

volume of stop mix. 5μl of each of the marker amplifications, prepared as above was analyzed on the same gel as the experimental samples. The samples were denatured at 95°C for 5 minutes immediately placed in an ice slurry and loaded onto the pre-run gels. The gels were run at 350V for the following periods: the multiply-spliced gel visualizing each transcript as a single band was run until the xylene cyanol (2^{nd} band reaches the gel bottom), the singly-spliced gel was run 30 minutes after the ^{2}nd dye as run off the bottom, the single-band multispliced species migrates with the xylene cyanol so the gel was run so that this band was close to the bottom, but not off. The actin, gag, and 9-27 gels were run approximately 15 minutes after the first dye had run off the gel (this allows the migration of the probe off the gel). The gels were dryed for 2 hours at 80°C, covered with fresh saran wrap and exposed to XAR film. The amount of radioactivity in these gels was quantitated in a phosphoimager or using the AMBIS radioanalytic imaging system. The levels of 9-27 expression was normalized to the levels of actin or other ubiquitous cellular RNA (such as glyhceraldehyde-3'-phosphate dehydrogenase, GAPDH or porphobilinogen deaminase, (PBGD).

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

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(1) GENERAL INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Thr Met Ile Thr Pro Ser Ala Gln Leu Thr Leu Thr Lys Gly Asn
1               5                  10                  15

Lys Ser Trp Ser Ser Thr Ala Val Ala Ala Ala Leu Glu Leu Val Asp
            20                  25                  30
```

Pro Pro Gly Cys Arg
35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Lys Glu Glu His Glu Val Ala Val Leu Gly Ala Pro Pro Ser
1 5 10 15

Thr Ile Leu Pro Arg Ser Thr Val Ile Asn Ile His Ser Glu Thr Ser
20 25 30

Val Pro Asp His Val Val Trp Ser Leu Phe Asn Thr Leu Phe Leu Asn
35 40 45

Trp Cys Cys Leu Gly Phe Ile Ala Phe Ala Tyr Ser Val Lys Ser Arg
50 55 60

Asp Arg Lys Trp Val Gly Asp Val Thr Gly Ala Gln Ala Tyr Ala Ser
65 70 75 80

Thr Ala Lys Cys Leu Asn Ile Trp Ala Leu Ile Leu Gly Ile Leu Met
85 90 95

Thr Ile Gly Phe Ile Leu Ser Leu Val Phe Gly Ser Val Thr Val Tyr
100 105 110

His Ile Met Leu Gln Ile Ile Gln Glu Lys Arg Gly Tyr
115 120 125

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 4..36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGGTTCCGC GTGGATCCCC GGGCCAGAAG ATG 33

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCCCGGGCC AGAAGATGCA CAAG 24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCACAAGCAC GTGCACTTTA TTGAA 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTCCCCAAAG CCAGAAGATG CA 22

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCACAAGCAC GTGCACTTTA TTGAA 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CATGAACCAC ATTGTGCAAA CCT 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAAACATATA CACTTTATTG AATG 24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATGAGTCAC ACTGTCCAAA CCT 23

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCAGAAACAC GTGCACTTTA T 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met His Lys Glu Glu His Glu Val Ala Val Leu Gly Ala Pro Pro Ser
1               5                  10                  15

Thr Ile Leu Pro Arg Ser Thr Val Ile Asn Ile His Ser Glu Thr Ser
            20                  25                  30

Val Pro Gly Ala Val Val Trp Ser Leu Phe Asn Thr Leu Phe Leu Asn
        35                  40                  45

Trp Cys Cys Leu Gly Phe Ile Ala Phe Ala Tyr Ser Val Lys Ser Arg
    50                  55                  60

Asp Arg Lys Trp Val Gly Asp Val Thr Gly Ala Gln Ala Tyr Ala Ser
65                  70                  75                  80

Thr Ala Lys Cys Leu Asn Ile Trp Ala Leu Ile Leu Gly Ile Leu Met
                85                  90                  95

Thr Ile Gly Phe Ile Leu Ser Leu Val Phe Gly Ser Val Thr Val Tyr
            100                 105                 110

His Ile Met Leu Gln Ile Ile Gln Glu Lys Arg Gly Tyr
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met His Lys Glu Glu His Glu Val Ala Val Leu Gly Ala Pro Pro Ser
1               5                  10                  15

Thr Ile Leu Pro Arg Ser Thr Val Ile Asn Ile His Ser Glu Thr Ser
            20                  25                  30

Val Pro Asp His Gly Ala Trp Ser Leu Phe Asn Thr Leu Phe Leu Asn
        35                  40                  45

Trp Cys Cys Leu Gly Phe Ile Ala Phe Ala Tyr Ser Val Lys Ser Arg
    50                  55                  60

Asp Arg Lys Trp Val Gly Asp Val Thr Gly Ala Gln Ala Tyr Ala Ser
65                  70                  75                  80

Thr Ala Lys Cys Leu Asn Ile Trp Ala Leu Ile Leu Gly Ile Leu Met
                85                  90                  95

Thr Ile Gly Phe Ile Leu Ser Leu Val Phe Gly Ser Val Thr Val Tyr
            100                 105                 110

His Ile Met Leu Gln Ile Ile Gln Glu Lys Arg Gly Tyr
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met His Lys Glu Glu His Glu Val Ala Val Leu Gly Ala Pro Pro Ser
1               5                  10                  15

Thr Ile Leu Pro Arg Ser Thr Val Ile Asn Ile His Ser Glu Thr Ser
            20                  25                  30

Val Pro Asp His Val Val Trp Ser Gly Ala Asn Thr Leu Phe Leu Asn
        35                  40                  45

Trp Cys Cys Leu Gly Phe Ile Ala Phe Ala Tyr Ser Val Lys Ser Arg
    50                  55                  60

Asp Arg Lys Trp Val Gly Asp Val Thr Gly Ala Gln Ala Tyr Ala Ser
65                  70                  75                  80

Thr Ala Lys Cys Leu Asn Ile Trp Ala Leu Ile Leu Gly Ile Leu Met
                85                  90                  95

Thr Ile Gly Phe Ile Leu Ser Leu Val Phe Gly Ser Val Thr Val Tyr
            100                 105                 110

His Ile Met Leu Gln Ile Ile Gln Glu Lys Arg Gly Tyr
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Met His Lys Glu Glu His Glu Val Ala Val Leu Gly Ala Pro Pro Ser
1               5                  10                  15

Thr Ile Leu Pro Arg Ser Gly Ala Ile Asn Ile His Ser Glu Thr Ser
            20                  25                  30

Val Pro Asp His Val Val Trp Ser Leu Phe Asn Thr Leu Phe Leu Asn
        35                  40                  45

Trp Cys Cys Leu Gly Phe Ile Ala Phe Ala Tyr Ser Val Lys Ser Arg
    50                  55                  60

Asp Arg Lys Trp Val Gly Asp Val Thr Gly Ala Gln Ala Tyr Ala Ser
65                  70                  75                  80

Thr Ala Lys Cys Leu Asn Ile Trp Ala Leu Ile Leu Gly Ile Leu Met
                85                  90                  95

Thr Ile Gly Phe Ile Leu Ser Leu Val Phe Gly Ser Val Thr Val Tyr
            100                 105                 110
```

His Ile Met Leu Gln Ile Ile Gln Glu Lys Arg Gly Tyr
115 120 125

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met His Lys Glu Glu His Glu Val Ala Val Leu Gly Ala Pro Pro Ser
1 5 10 15

Thr Ile Leu Pro Arg Ser Thr Val Ile Asn Ile His Ser Glu Thr Ser
20 25 30

Val Pro Asp His Val Val Trp Ser Leu Phe Asn Thr Leu Phe Leu Asn
35 40 45

Trp Cys Cys Leu Gly Phe Ile Ala Phe Ala Tyr Ser Val Lys Ser Gly
50 55 60

Ala Arg Lys Trp Val Gly Asp Val Thr Gly Ala Gln Ala Tyr Ala Ser
65 70 75 80

Thr Ala Lys Cys Leu Asn Ile Trp Ala Leu Ile Leu Gly Ile Leu Met
85 90 95

Thr Ile Gly Phe Ile Leu Ser Leu Val Phe Gly Ser Val Thr Val Tyr
100 105 110

His Ile Met Leu Gln Ile Ile Gln Glu Lys Arg Gly Tyr
115 120 125

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met His Lys Glu Glu His Glu Val Ala Val Leu Gly Ala Pro Pro Ser
1 5 10 15

Thr Ile Leu Pro Arg Ser Thr Val Ile Asn Ile His Ser Glu Thr Ser
20 25 30

Val Pro Asp His Val Val Trp Ser Leu Phe Asn Thr Leu Phe Leu Asn
35 40 45

Trp Cys Cys Leu Gly Phe Ile Ala Phe Ala Tyr Ser Val Lys Ser Arg
50 55 60

Asp Arg Lys Trp Val Gly Asp Val Thr Gly Ala Gln Ala Tyr Ala Ser
65 70 75 80

Thr Ala Lys Cys Leu Asn Ile Trp Ala Leu Ile Leu Gly Ile Leu Met
85 90 95

Thr Ile Gly Phe Gly Ala Ser Leu Val Phe Gly Ser Val Thr Val Tyr
100 105 110

His Ile Met Leu Gln Ile Ile Gln Glu Lys Arg Gly Tyr
115 120 125

WHAT IS CLAIMED IS:

1. A method of inhibiting a viral infection in a host comprising:

administering to the host in an amount effective to inhibit the infection a polypeptide capable of binding to a viral Rev responsive element, which polypeptide is encoded by a first interferon-induced 1-8 gene family member, or a mutant thereof.

2. The method of claim 1, wherein the viral infection is a retroviral infection.

3. The method of claim 2, wherein the retroviral infection is caused by HIV virus.

4. The method of claim 3, wherein the HIV virus is HIV-1.

5. The method of claim 2, wherein the retroviral infection is caused by HTLV-I.

6. The method of claim 1, wherein the polypeptide is RBP927.

7. The method of claim 1, wherein the polypeptide is SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14.

8. The method of claim 1, wherein the polypeptide is administered to the host in multiple doses.

9. The method of claim 1, further comprising administering a compound having antiviral activity to the host.

10. The method of claim 9, wherein the compound is a polypeptide encoded by a second interferon-inducible gene 1-8 family member, or a mutant thereof.

11. The method of claim 10, wherein the first interferon-inducible gene 1-8 family member is RBP927 and the second interferon-inducible gene 1-8 family member is 1-8U.

12. The method of claim 9, wherein the compound is a transdominant Rev protein.

13. The method of claim 12, wherein the transdominant Rev protein is RevBL.

14. A method of inhibiting structural protein expression of a virus in human cells comprising:

administering to the cells in an amount effective to inhibit expression of said protein a polypeptide capable of binding to a Rev responsive element of the retrovirus, which polypeptide is encoded by a first interferon-induced 1-8 gene family member, or mutant thereof.

15. The method of claim 14, wherein the virus is a retrovirus.

16. The method of claim 14, wherein the interferon-induced 1-8 gene family member encoding the polypeptide is gene 9-27 or gene 1-8U.

17. The method of claim 15, wherein the polypeptide is RBP927, SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14, and the retrovirus is HIV-1 or HTLV-I.

18. The method of claim 14, further comprising administering an antiviral compound to the cells.

19. The method of claim 18, wherein the antiviral compound is a peptide encoded by a second interferon-inducible gene 1-8 family member, or mutant thereof.

20. The method of claim 18, wherein the antiviral compound is RevBL.

21. A pharmaceutical composition, comprising a polypeptide capable of binding to a retroviral Rev-responsive element, which polypeptide is encoded by a first interferon-induced 1-8 gene family member or mutant thereof, and a pharmaceutically acceptable carrier.

22. The pharmaceutical composition of claim 21, wherein the first interferon-induced 1-8 gene family member encoding the polypeptide is gene 9-27 or 1-8U.

23. A eukaryotic expression vector comprising a member of the interferon-induced 1-8 gene family, or mutant thereof, operably linked to a retroviral promoter.

24. A vector of claim 23, wherein the member of the interferon-induced 1-8 gene family is gene 9-27.

25. A method of inhibiting viral infection in host cells comprising:

transfecting the host cells with a eukaryotic expression vector of claim 23.

26. A method of claim 25, further comprising inducing transcription of the interferon-induced 1-8 gene family member in the host cell.

27. A method of claim 25, further comprising transfecting the host cell with a eukaryotic expression vector comprising a nucleic acid sequence encoding a transdominant Rev protein.

28. A method for assessing the efficacy of interferon therapy in a patient comprising:

quantitating the level of a expression product of an interferon-induced 1-8 gene family member in cells of the patient during therapy;

comparing the level of the transcription product during therapy to the level of the transcription product in cells not exposed to interferon therapy; and

determining the efficacy of the interferon therapy therefrom.

29. A method of claim 28, wherein the expression product is mRNA.

30. A method of claim 28, wherein the expression product is a protein.

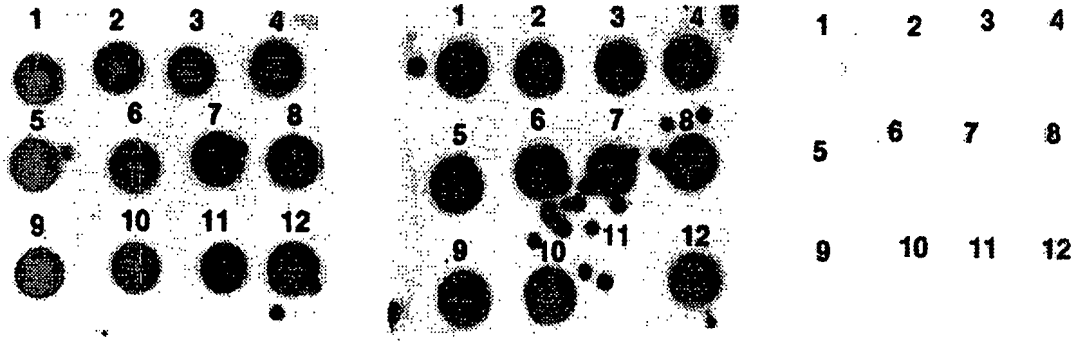


FIG. 1A.

Fig. 1B

RBP927 PROTEIN:

```
    |  10     |  20     |  30     |  40
MHKEEHEVAV LGAPPSTILP RSTVINIHSE TSVPDHVVWS

    |  50     |  60     |  70     |  80
LFNTLFLNWC CLGFIAFAYS VKSRDRKMVG DVTGAQAYAS

    | 110     | 120     | 130     | 140   145
TAKCLNIWAL ILGILMTIGF ILSLVFGSVT VYHIMLQIIQ EKRGY
```

β-gal-RBP927 HYBRID PROTEIN IN CLONE #11:

```
    |  10     |  20     |  30      37
MTMITPSAQL TLTKGNKSWS STAVAAALEL VDPPGCR...--RBP927
```

Fig. 1C

GST-RBP927 HYBRID PROTEIN EXPRESSED IN VECTOR pGEX-2T:

```
GST--CTG GTT CCG CGT GGA TCC CCG GGC CAG AAG ATG--RBP927
     leu val pro arg gly ser pro gly gln lys met
                  ↑
     Thrombin cleavage site
```

Fig. 1D

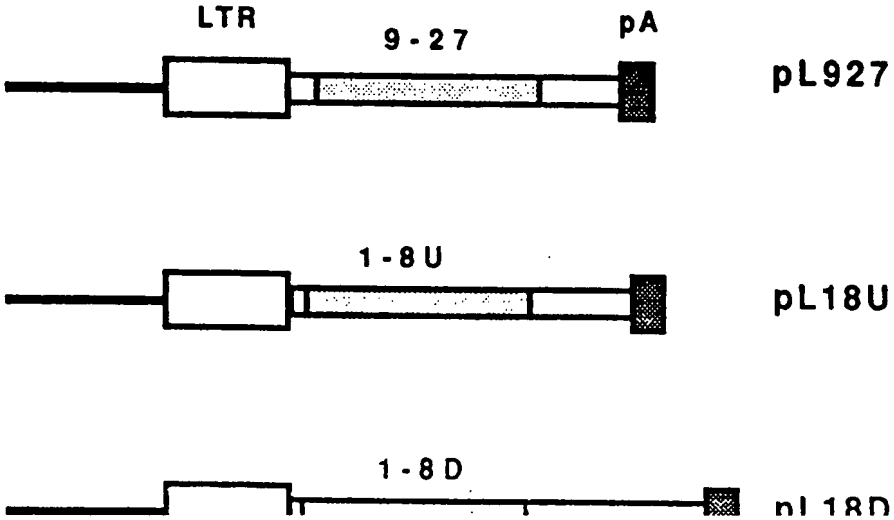


Fig. 2A

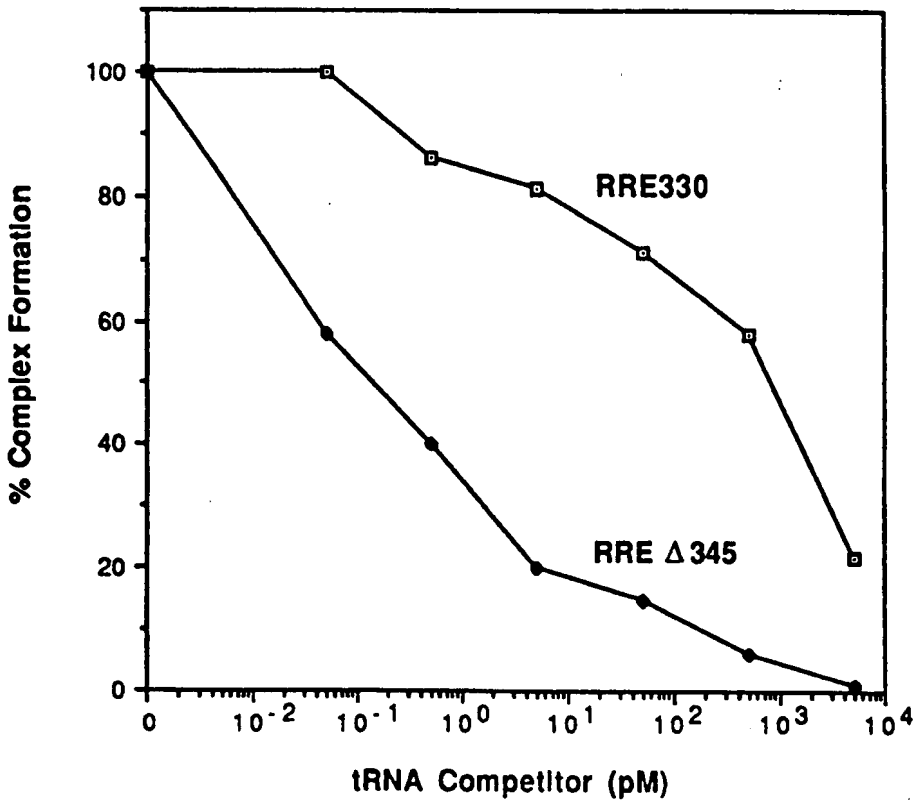
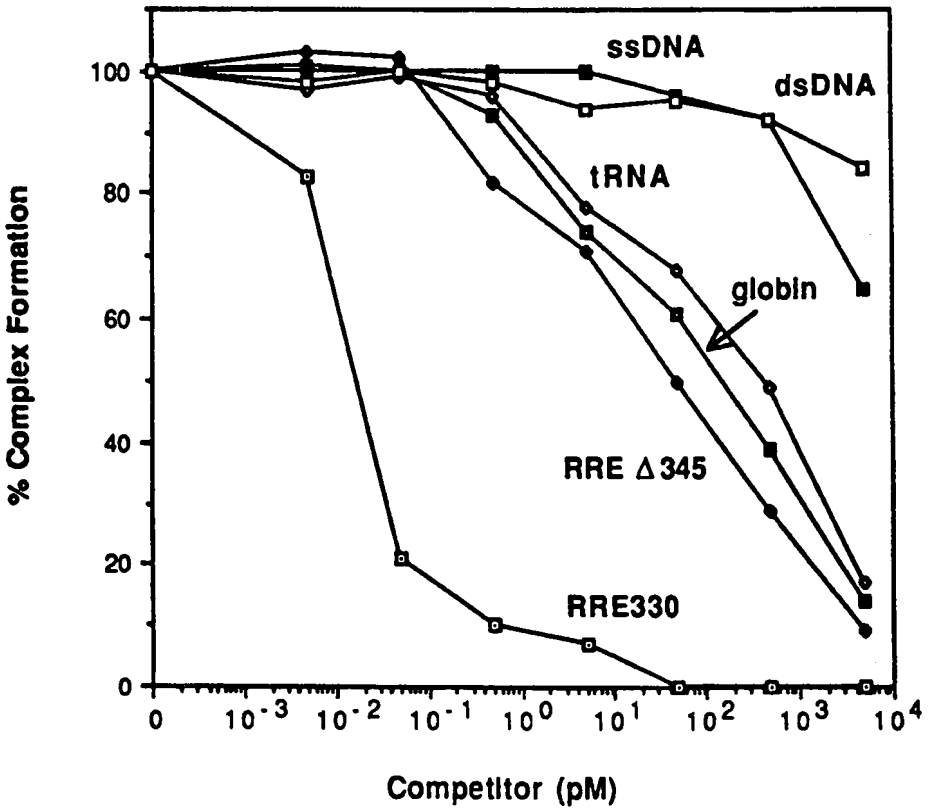

% Complex Formation
100
80
60
40
20
0
RRE330
RRE Δ345
0
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
tRNA Competitor (pM)

Fig. 2B


ssDNA
dsDNA
tRNA
globin
RRE Δ345
RRE330
% Complex Formation
Competitor (pM)
100
80
60
40
20
0
0
10-3
10-2
10-1
100
101
102
103
104

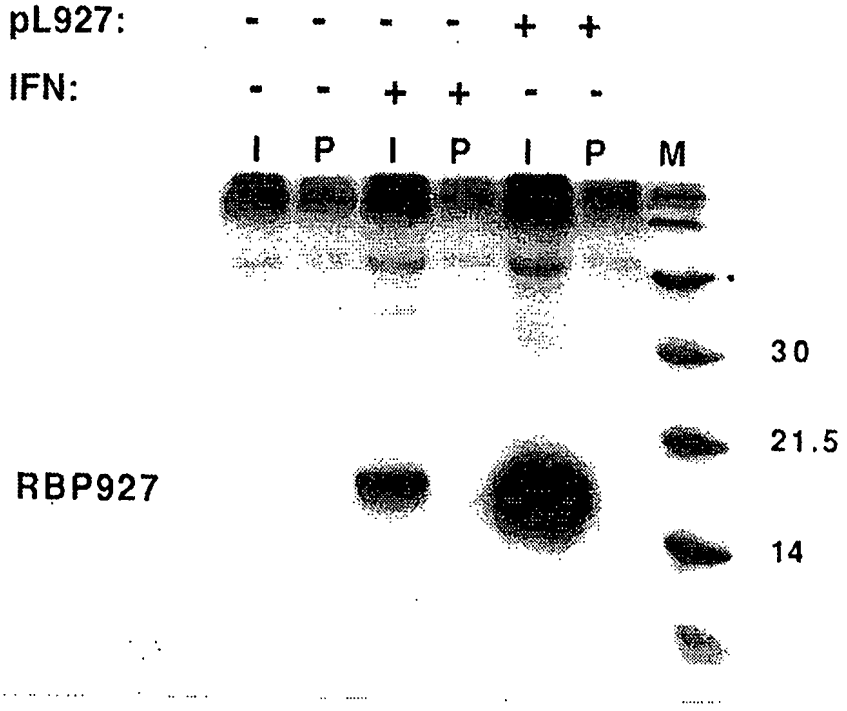


FIG. 3A.

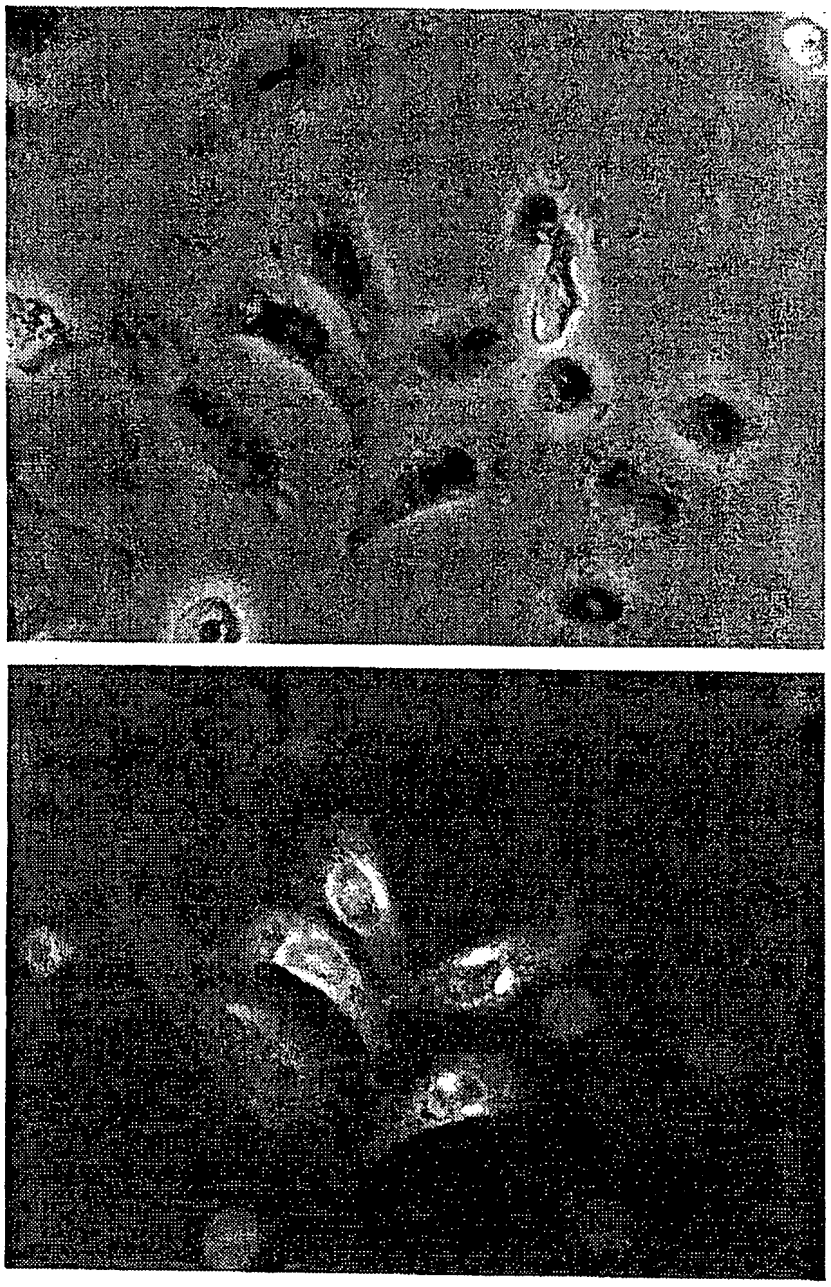


FIG. 3B.

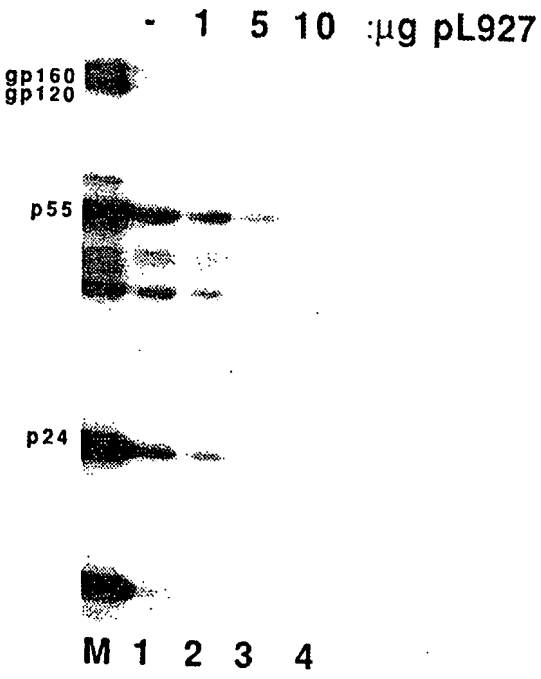
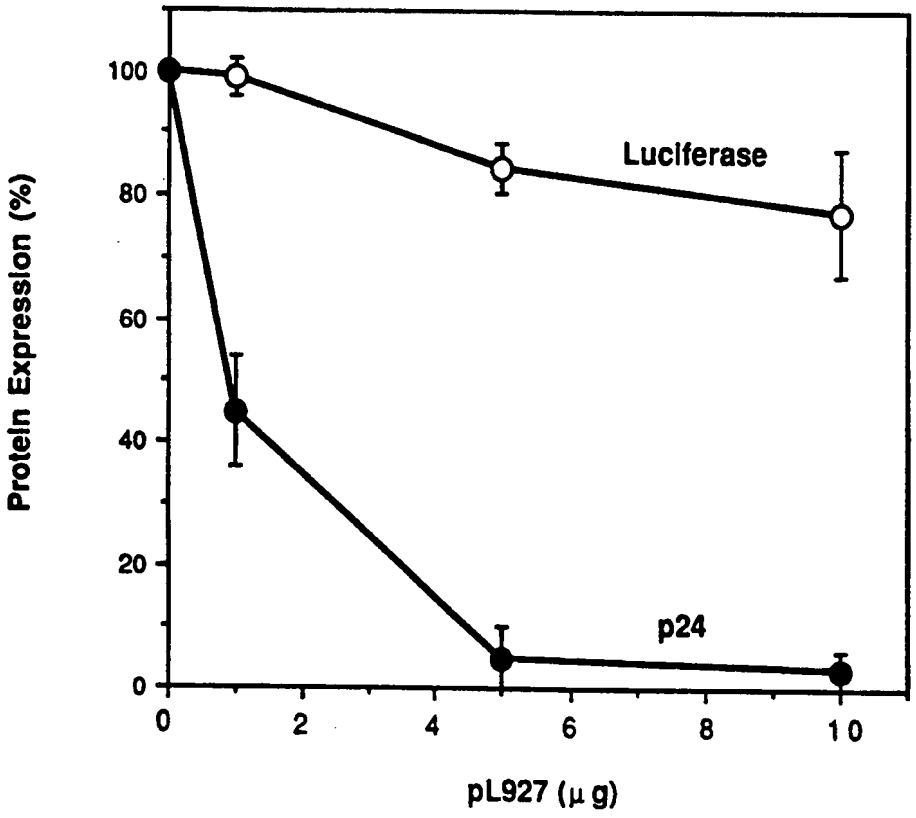


FIG. 4A.

Fig. 4B


Protein Expression (%)
100
80
60
40
20
0
0
2
4
6
8
10
pL927 (μg)
Luciferase
p24

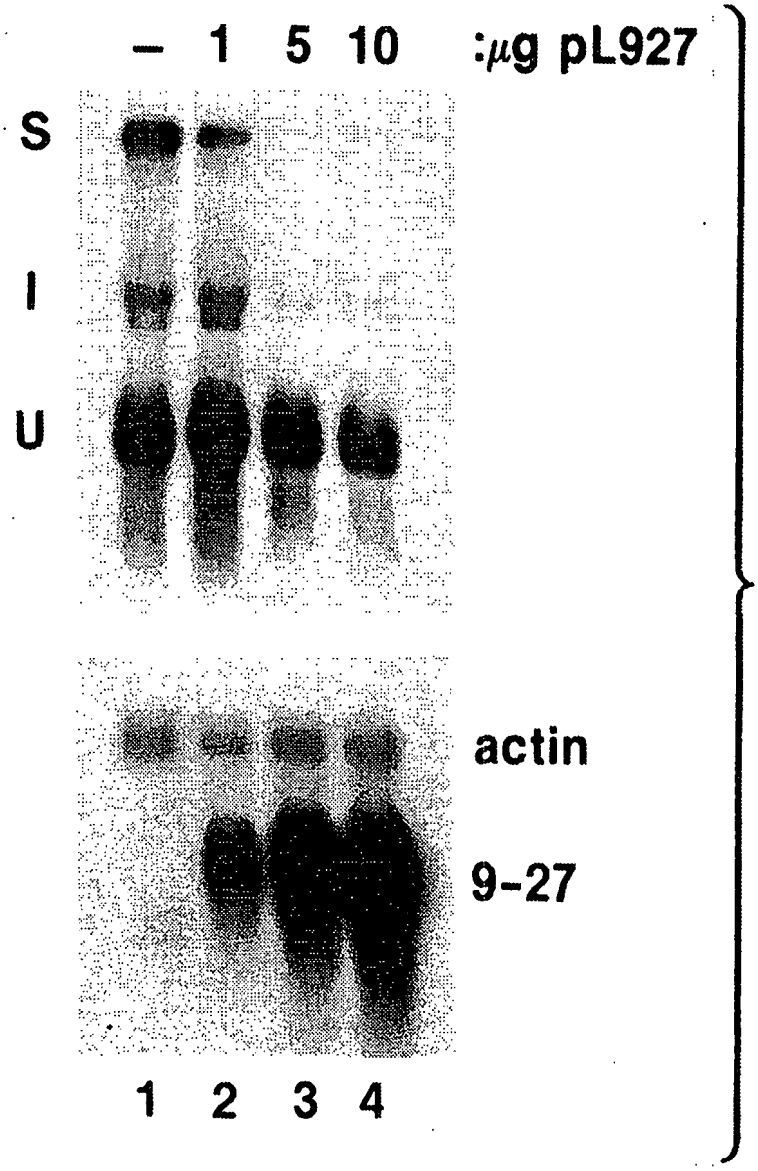

– 1 5 10 :μg pL927
S
I
U
actin
9-27
1 2 3 4

FIG. 4C.

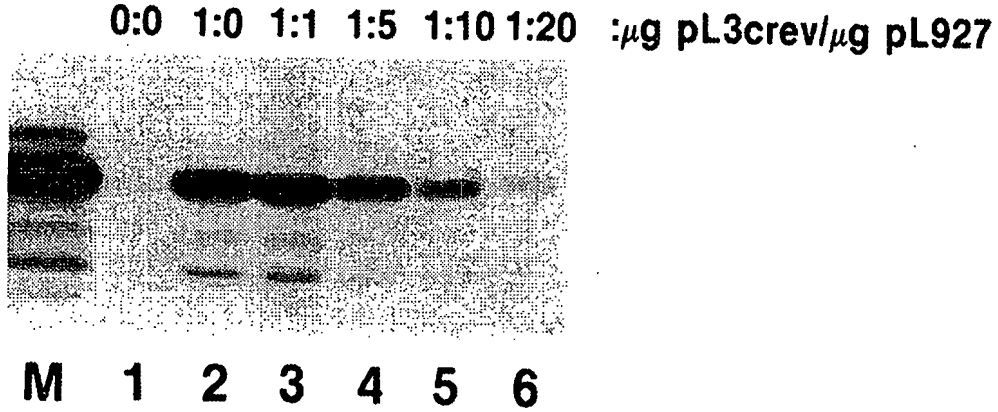

0:0 1:0 1:1 1:5 1:10 1:20 :μg pL3crev/μg pL927
M 1 2 3 4 5 6

FIG. 5A.

Fig. 5B

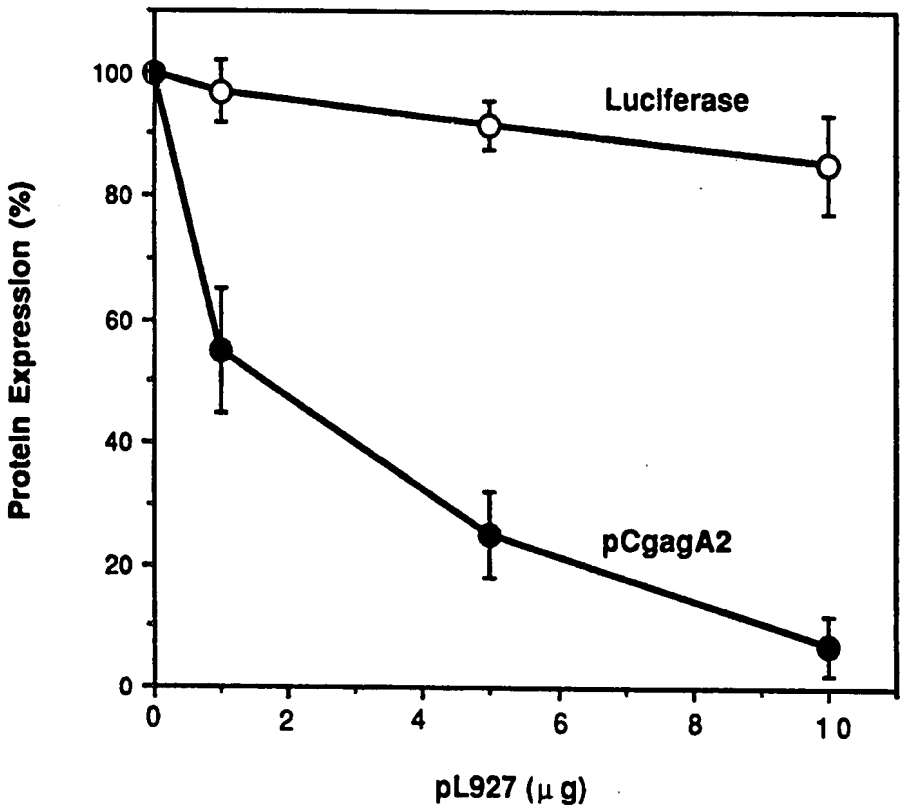
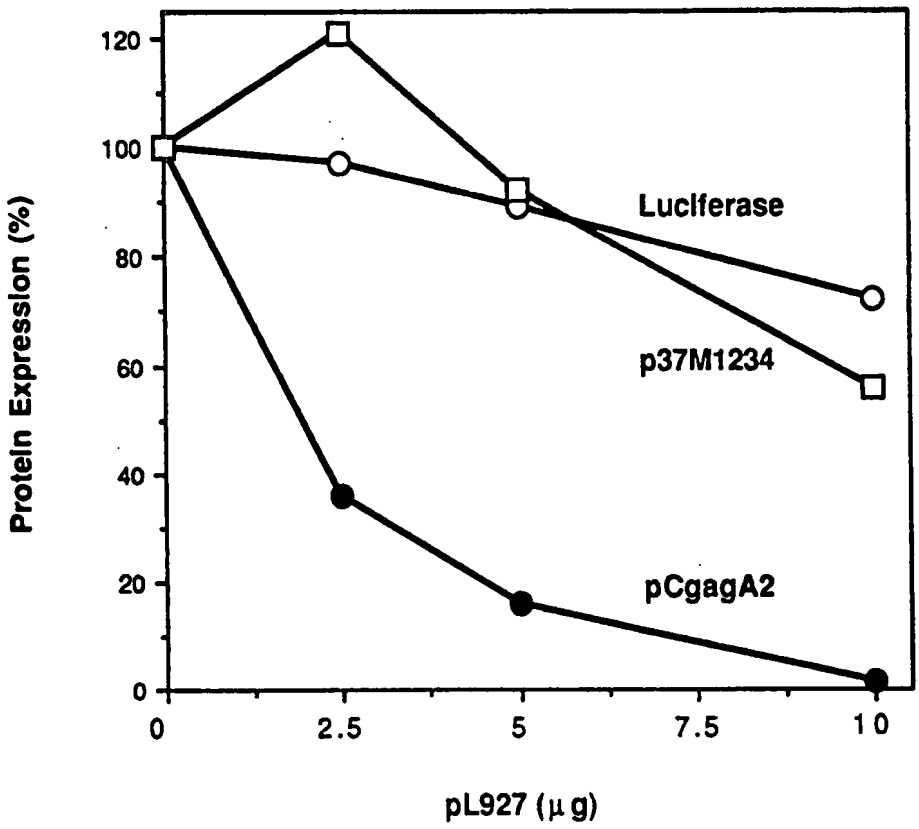

Protein Expression (%)
100
80
60
40
20
0
0
2
4
6
8
10
pL927 (μg)
Luciferase
pCgagA2

Fig. 6A


Protein Expression (%)
120
100
80
60
40
20
0
0
2.5
5
7.5
10
pL927 (μg)
Luciferase
p37M1234
pCgagA2

RBP927 INHIBITS ONLY REV-DEPENDENT PROTEIN EXPRESSION

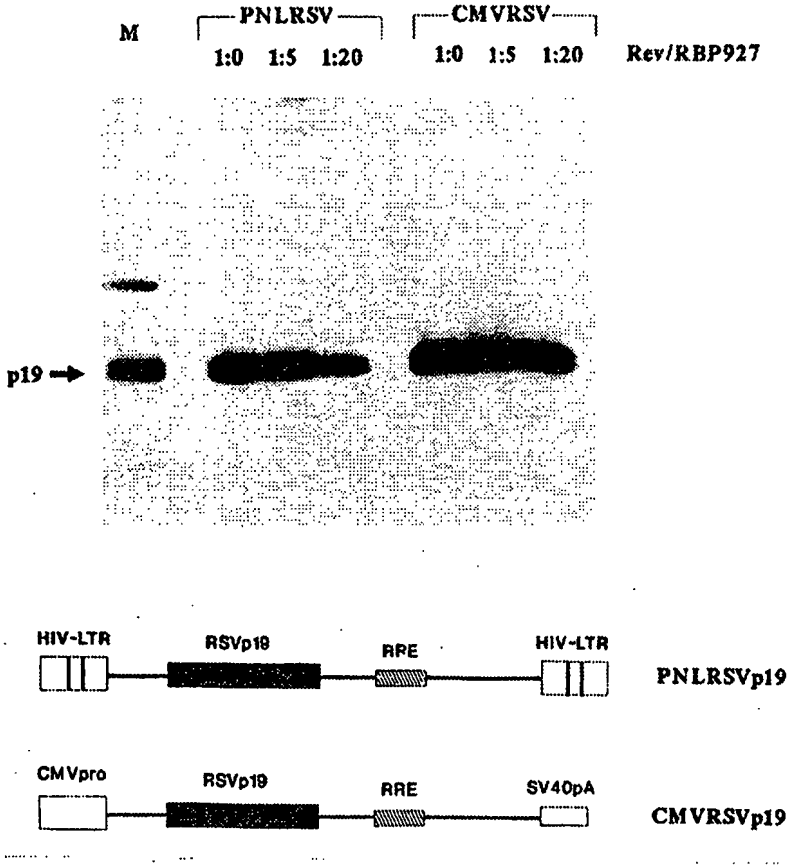


FIG. 6B.

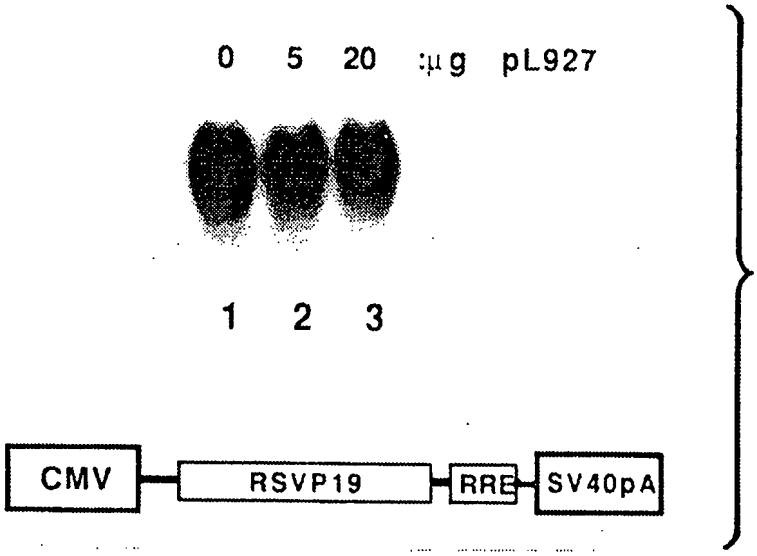

0 5 20 :µg pL927
1 2 3
CMV
RSVP19
RRE
SV40pA

FIG. 6C.

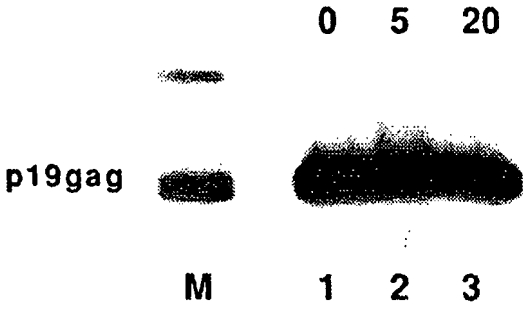

0 5 20 :µg pL927
p19gag
M 1 2 3

FIG. 6D.

Fig. 7A

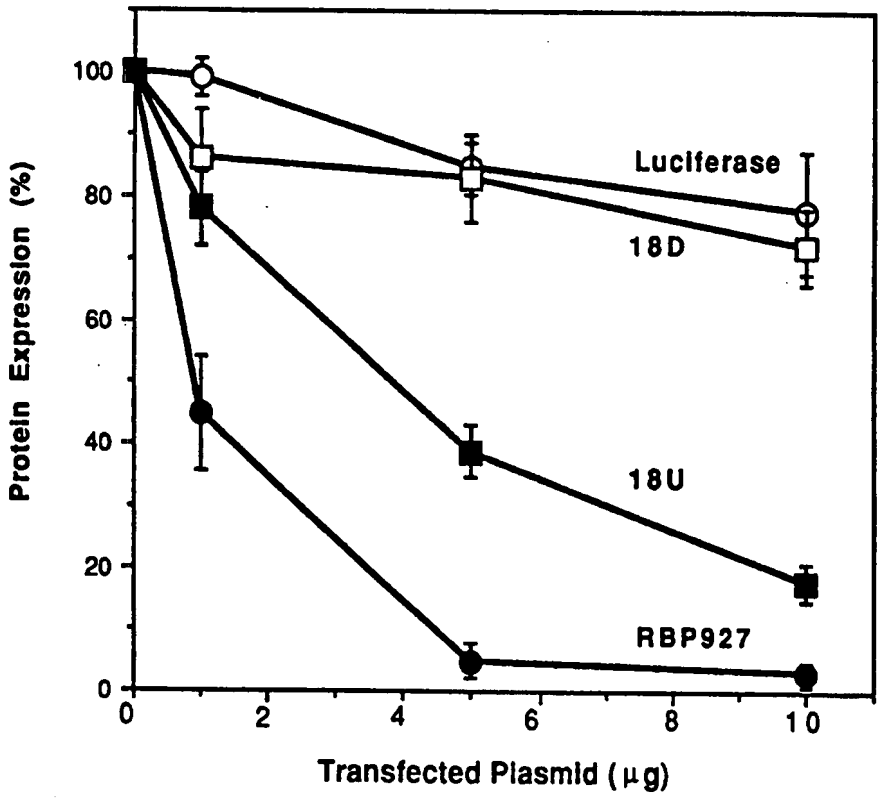
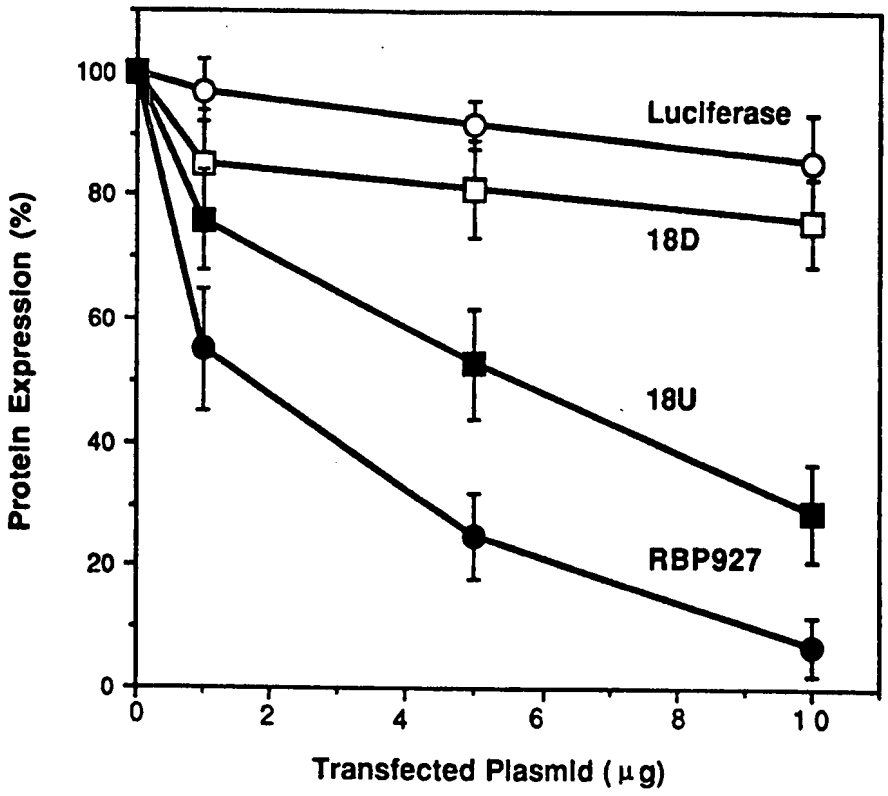

Protein Expression (%)
100
80
60
40
20
0
0
2
4
6
8
10
Transfected Plasmid (μg)
Luciferase
18D
18U
RBP927

Fig. 7B


Protein Expression (%)
100
80
60
40
20
0
0
2
4
6
8
10
Transfected Plasmid (μg)
Luciferase
18D
18U
RBP927

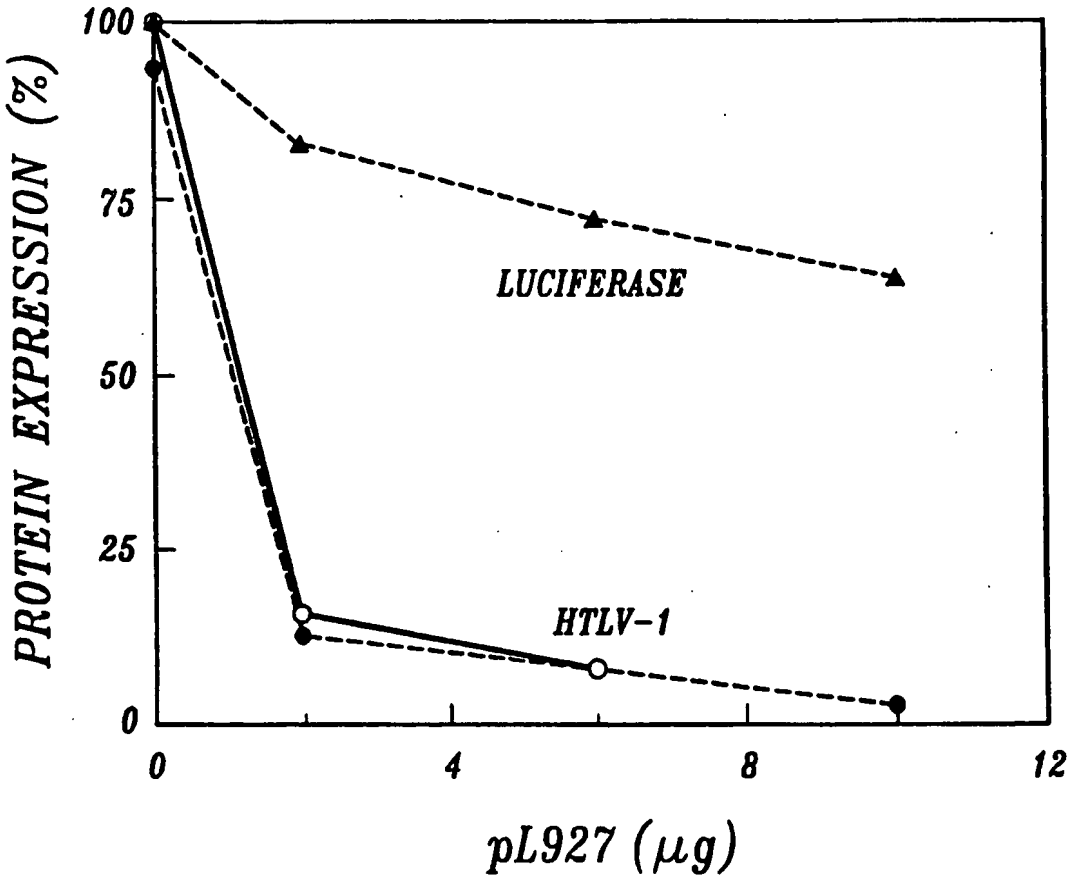
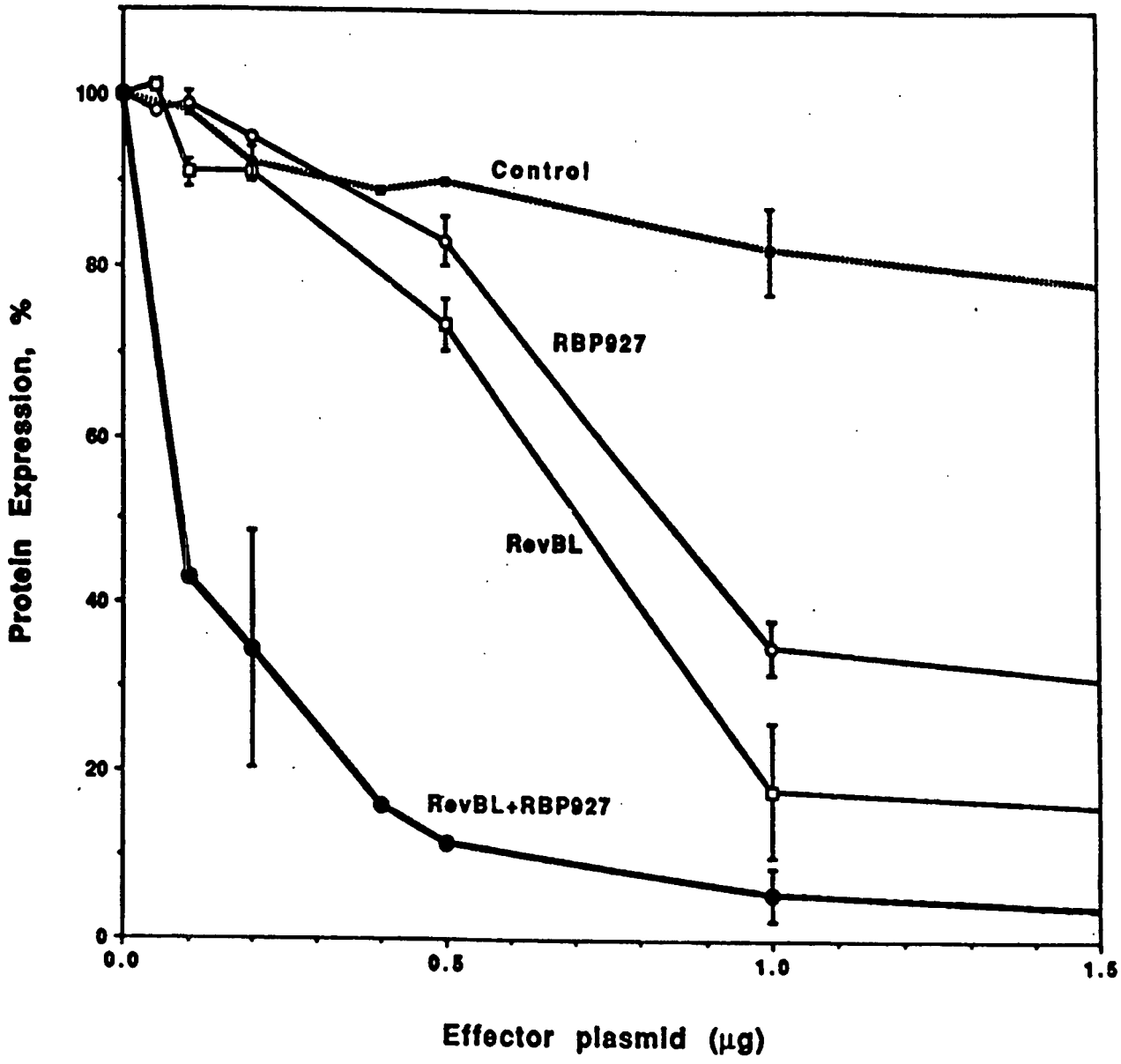

100
75
50
25
0
PROTEIN EXPRESSION (%)
LUCIFERASE
HTLV-1
0
4
8
12
pL927 (μg)

FIG. 8.

Fig. 9


Protein Expression, %
100
80
60
40
20
0
0.0
0.5
1.0
1.5
Effector plasmid (μg)
Control
RBP927
RevBL
RevBL+RBP927

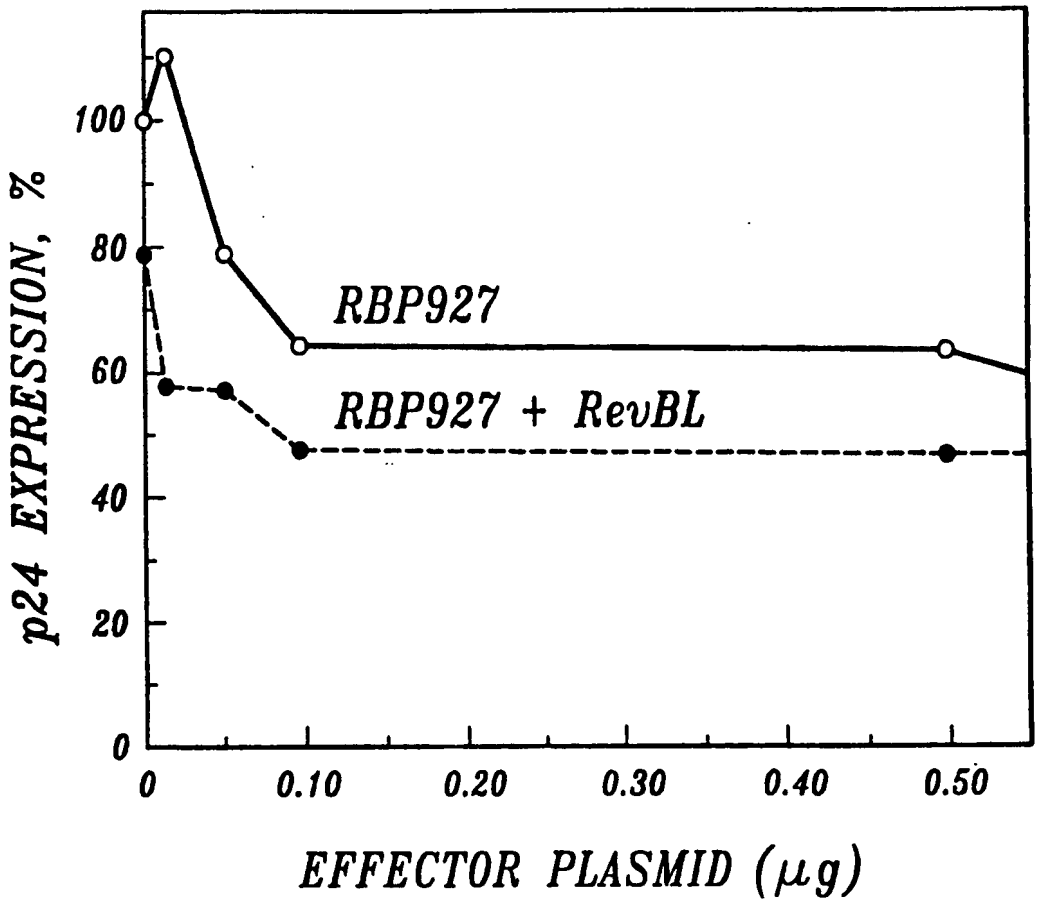

p24 EXPRESSION, %
100
80
60
40
20
0
RBP927
RBP927 + RevBL
0
0.10
0.20
0.30
0.40
0.50
EFFECTOR PLASMID (μg)

FIG. 10.

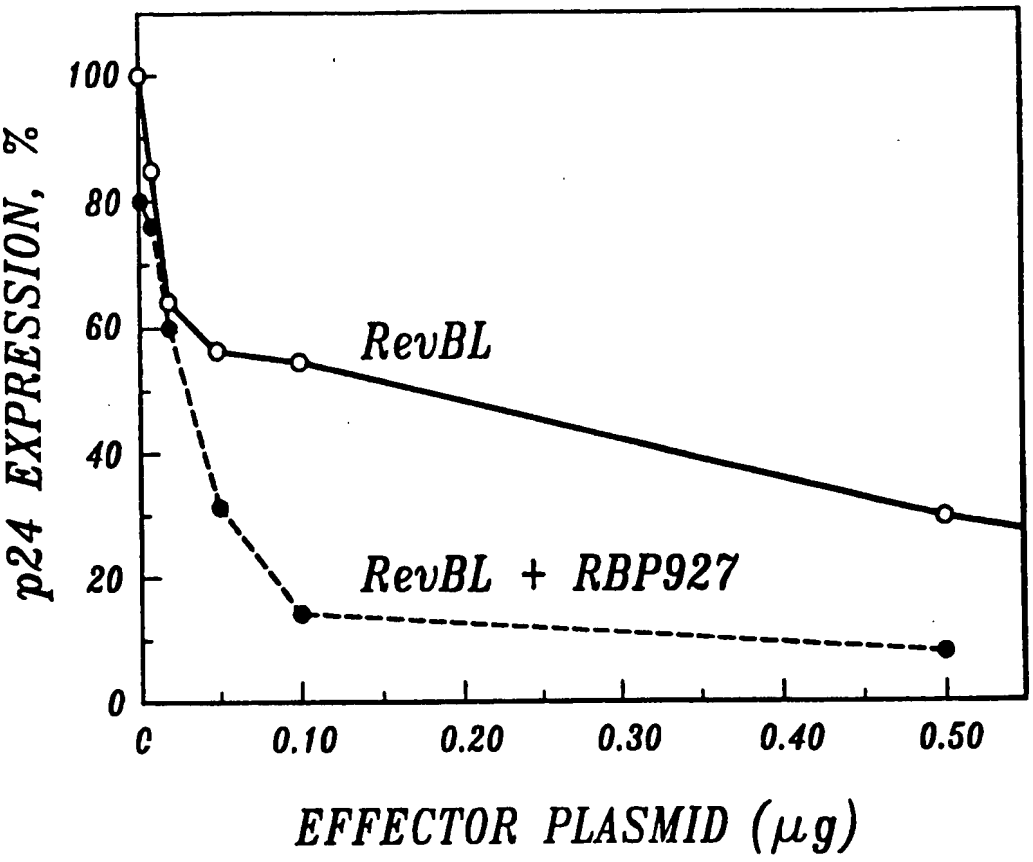

p24 EXPRESSION, %
100
80
60
40
20
0
0
0.10
0.20
0.30
0.40
0.50
RevBL
RevBL + RBP927
EFFECTOR PLASMID (μg)

FIG. 11.

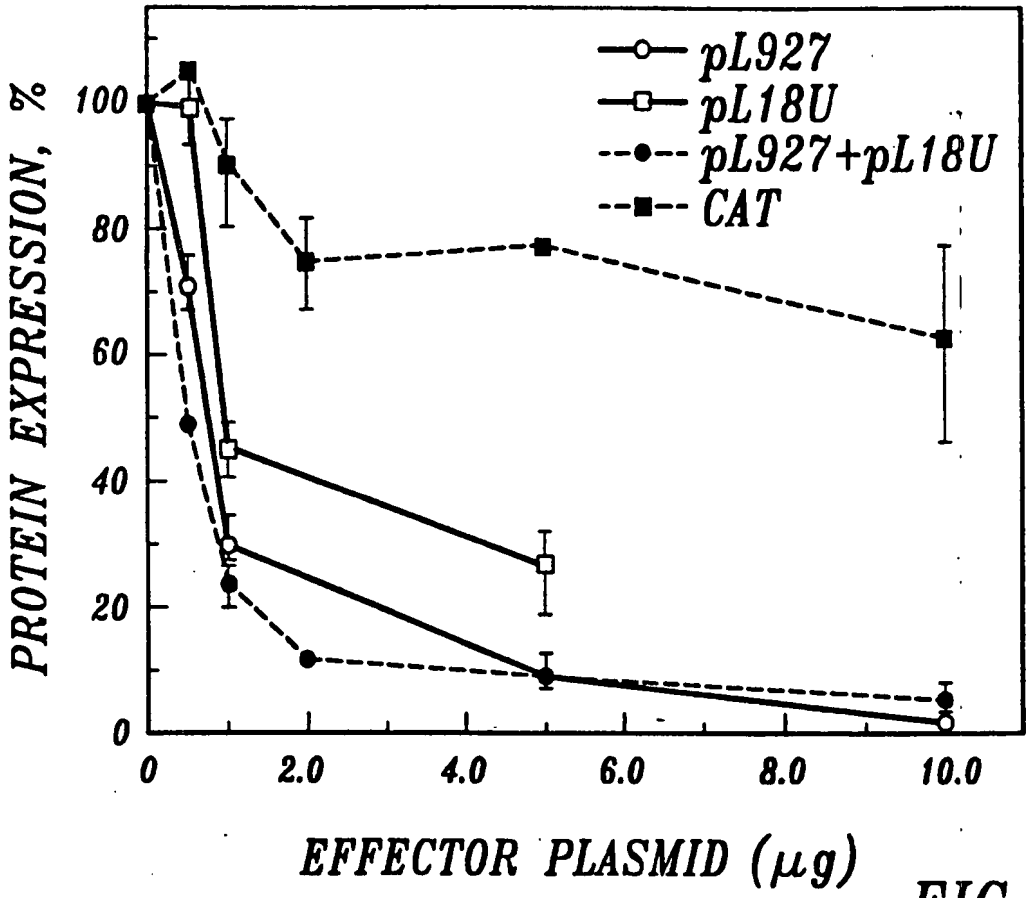

pL927
pL18U
pL927+pL18U
CAT
PROTEIN EXPRESSION, %
100
80
60
40
20
0
0
2.0
4.0
6.0
8.0
10.0
EFFECTOR PLASMID (μg)

FIG. 12A.

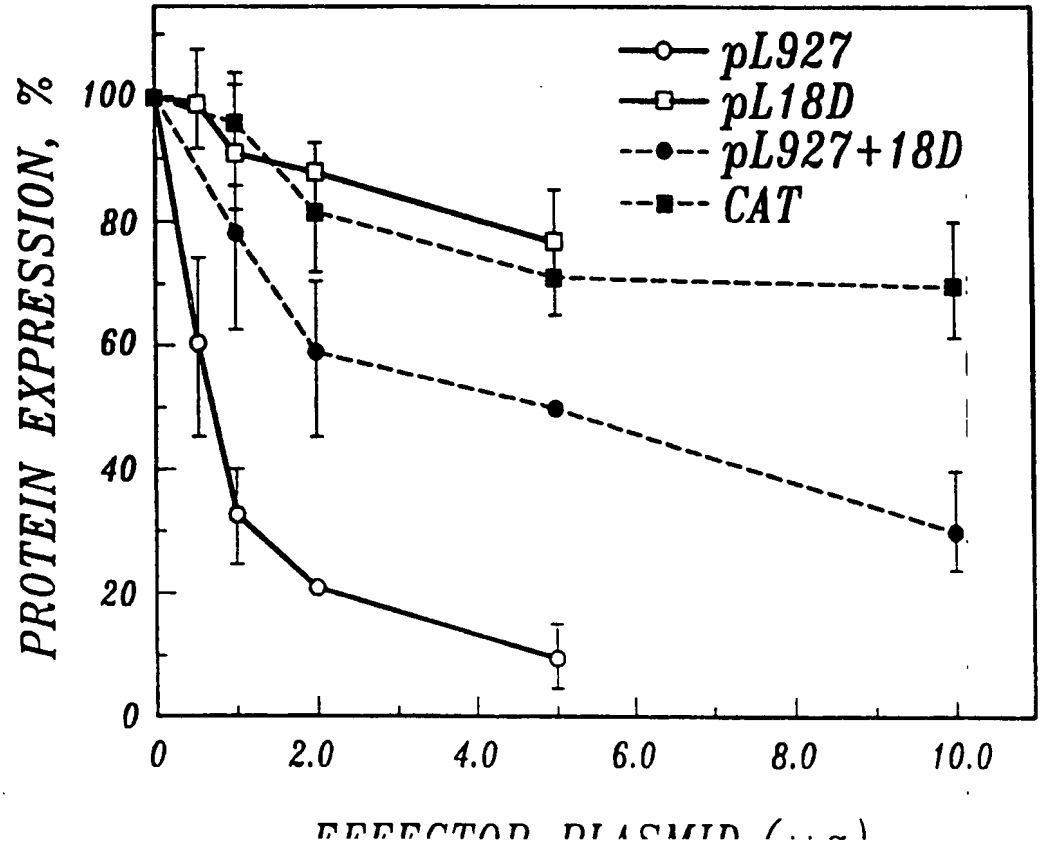

pL927
pL18D
pL927+18D
CAT
PROTEIN EXPRESSION, %
100
80
60
40
20
0
0
2.0
4.0
6.0
8.0
10.0

Fig. 13

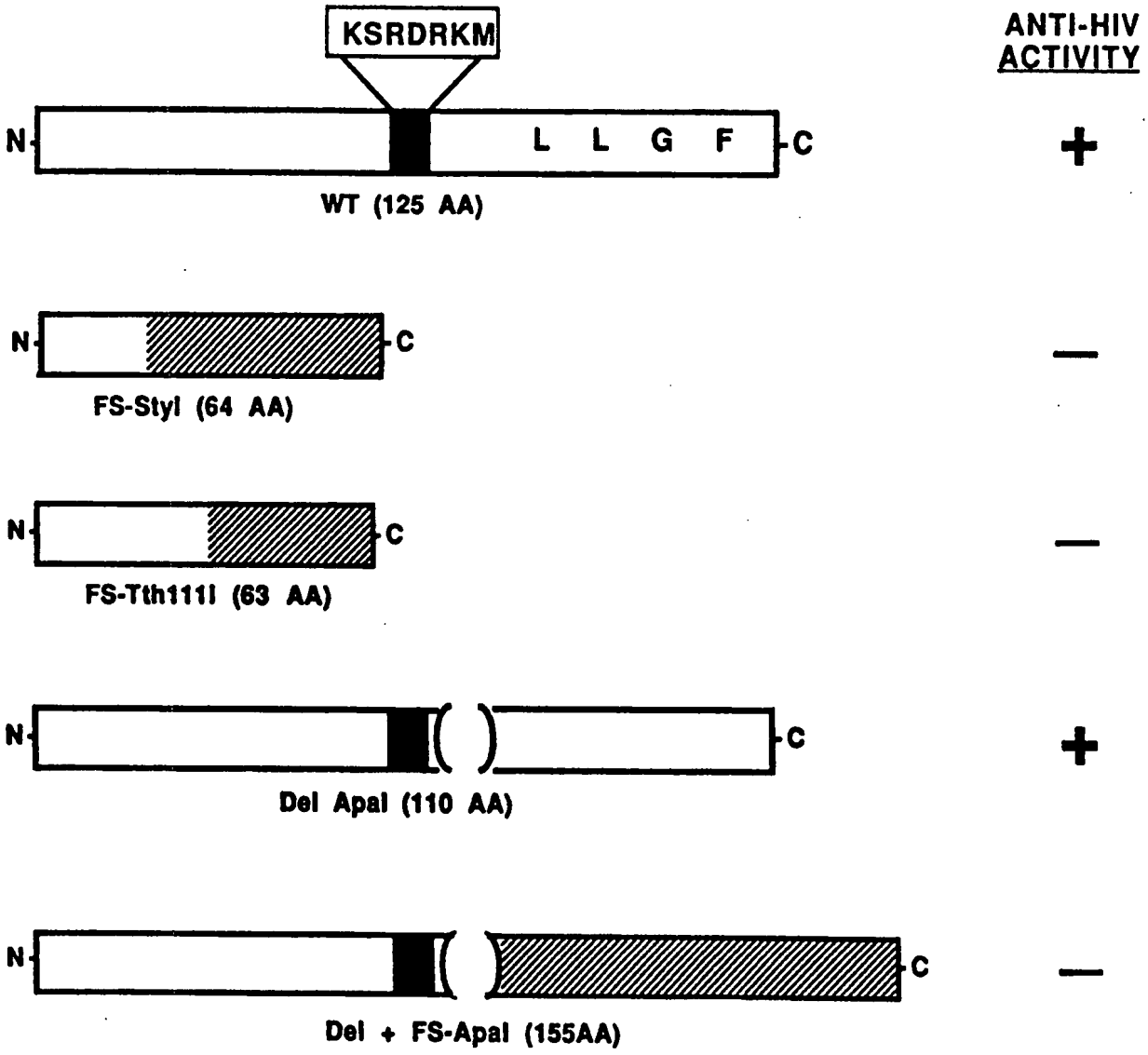

KSRDRKM
ANTI-HIV ACTIVITY
N
L L G F
C
WT (125 AA)
+
N
C
FS-StyI (64 AA)
—
N
C
FS-Tth111I (63 AA)
—
N
C
Del ApaI (110 AA)
+
N
C
Del + FS-ApaI (155AA)
—

Fig. 14

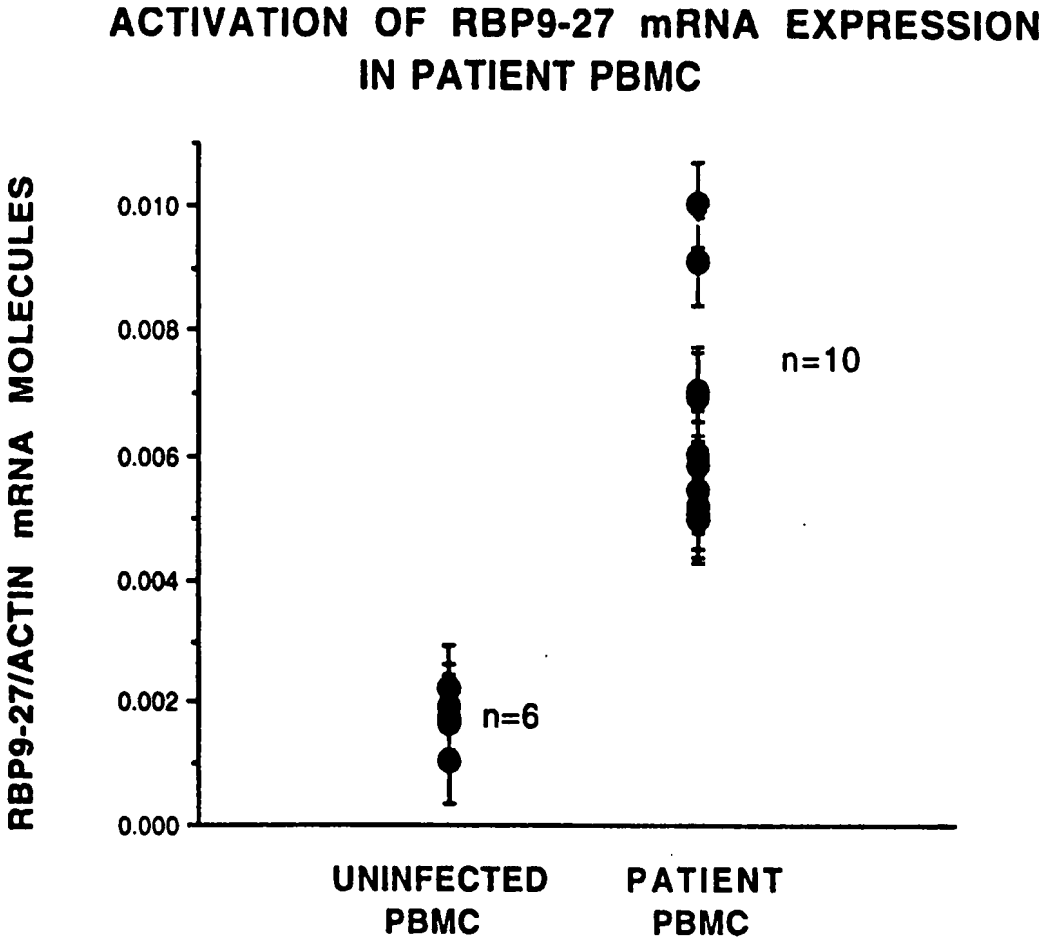
9-27 MUTAGENESIS

```
    5    10    15    20    25    30    35    40    45    50    55    60
    !    !     !     !     !     !     !     !     !     !     !     !
MHKEEHEVAVLGAPPSTILPRSTVINIHSETSVPDHVVWSLFNTLFLNWCCLGFIAFAYS

   65    70    75    80    85    90    95   100   105   110   115   120   125
    !    !     !     !     !     !     !     !     !     !     !     !     !
VKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTIGFILSLVFGSVTVYHIMLQIIQEKRGY
```

MUTANT	AMINO ACID	HIV INHIBITION
WT	-	+
1	(23/24)	nd
2	(31/32)	+
3	(35/36)	++
4	(37/38)	++
5	(41/42)	++
6	(50/51)	nd
7	(62/63)	-
8	(64/65)	+
9	(66/67)	nd
10	(84/85)	nd
11	(91/92)	nd
12	(94/95)	nd
13	(101/102)	+

Fig. 15


ACTIVATION OF RBP9-27 mRNA EXPRESSION IN PATIENT PBMC
RBP9-27/ACTIN mRNA MOLECULES
0.010
0.008
0.006
0.004
0.002
0.000
n=10
n=6
UNINFECTED PBMC
PATIENT PBMC

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/06829

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 A61K37/02 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K C12N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 86 , February 1989 , WASHINGTON US pages 840 - 844 REID, L. ET AL. 'A single DNA response element can confer inducibility by both alpha and gamma interferons' cited in the application see the whole document ---	1-27
A	EUROPEAN JOURNAL OF BIOCHEMISTRY vol. 199, no. 2 , July 1991 , BERLIN, DE pages 417 - 423 LEWIN, A. ET AL. 'Molecular analysis of a human interferon-inducible gene family' cited in the application see the whole document --- -/--	1-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 November 1993	16. 12. 93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Andres, S

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUCLEIC ACIDS RESEARCH vol. 18, no. 8 , 1990 , ARLINGTON, VIRGINIA US pages 2037 - 2044 MERMER, B. ET AL. 'Identification of trans-dominant HIV-1 rev protein mutants by direct transfer of bacterially produced proteins into human cells' cited in the application see the whole document	12,13, 20,27
A	EP,A,0 242 329 (CIBA-GEIGY A.G.) 21 October 1987 see claims; figures	
P,X	SCIENCE vol. 259 , 26 February 1993 , LANCASTER, PA US pages 1314 - 1318 CONSTANTOULAKIS, P. ET AL. 'Inhibition of rev-mediated HIV-1 expression by an RNA binding protein encoded by the interferon-inducible 9-27 gene' cited in the application see the whole document	1-4,6, 14-16

INTERNATIONAL SEARCH REPORT

[redacted]tional application No.
PCT/US93/06829

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-20, 25-30 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/ composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found muluple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 93/06829

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0242329	21-10-87	AU-B- 608216	28-03-91
		AU-A- 7151087	22-10-87
		JP-A- 62270599	24-11-87
		US-A- 5198350	30-03-93